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SOME CHROMOSOME COMPLEMENTS IN THE CACTACEAE AND A STUDY OF MEIOSIS IN ECHINOCEREUS PAPILLOSUS[1]

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(WITH PLATES I, II AND TEXT FIGURES)

Introduction

The cytology of the Cactaceae is of particular interest because of the development in the family of certain morphological characters which sharply separate it from all others. As a whole, this family contains well over 1000 species in 124 genera. The material for this paper was taken entirely from the tribe Cereeae. Since the evidence indicates that this is the most highly evolved section, it seems reasonable to suppose that as wide a range of variation in chromosome numbers will be found here as in either of the other two tribes.

This paper reports briefly the results of a cytological study of forty-six species, including two forms and one species thought to be a hybrid. The chromosome complements of these species are described and their relationships to the taxonomy and phylogeny of the group are discussed. It is hoped that this contribution, supplemented by future work, may determine whether there are cytological peculiarities associated with taxonomic relationships already established on the basis of morphological characters. More particularly, series of polyploid and aneuploid chromosome numbers may be built up which may establish possible lines of evolution and points at which new species have arisen.

[1] Papers from the Department of Botany, University of Michigan, no. 614.

Since there was no account of meiosis in the Cactaceae, a careful study was made of microsporogenesis in *Echinocereus papillosus* Linke, a representative of one of the more complex genera.

TISCHLER (28) lists two counts of chromosomes in the *Neomammillaria*, one by JARETZKY (16) and the other by ISHII (14), both giving the haploid number as eleven. SUGIURA (25), in his list of chromosome numbers in the angiosperms, reports the diploid number of twenty-four in two species of *Neomammillaria* and one of *Zygocactus* (*Epiphyllum*). *Opuntia brasiliensis* is reported by JOHANSEN (17) as having a diploid number of twenty-two. STOCKWELL (24) gives haploid numbers of nine, eleven, twenty-two, and thirty-three from seventeen types. The brief paper by JOHANSEN and the longer one by STOCKWELL, both concerned with chromosomes as found in root tips, offer the first substantial contribution to the knowledge of the chromosomes of this group.

Material and methods

The material for this study was derived from a number of sources. Part of it has been under cultivation for some time at the University of Michigan Botanical Garden and came originally from the collections of the Missouri Botanical Garden. Most of the Central American species, with those from Tamaulipas, Mexico, were collected by Prof. H. H. BARTLETT. Those from the San Luis Potosi area were obtained by Dr. CYRUS LUNDELL. The remainder were collected by Dr. ELZADA CLOVER and the writer in the lower Rio Grande Valley, Texas.

Material was determined throughout from the monograph on the Cactaceae by BRITTON and ROSE (3).

The species which bloomed freely provided ample material for the study of the chromosomes of pollen mother cells during microsporogenesis. Counts were obtained in other species from root tips, particularly from certain species with characteristic aerial roots.

Neomammillaria and related genera were difficult to handle because the buds did not emerge at the bases of the papillae until almost mature. It was necessary to remove them as soon as the wool in which they were imbedded began to push out. At this time they

could be lifted out by inserting a small scalpel at the base of the papilla and prying gently.

To determine the stage of meiosis in buds, anthers were examined in aceto-carmine smears. It was found that meiotic divisions were most numerous from 10 A.M. until 2 P.M. Diakinesis stages were most frequently found just before noon. To obtain a successful smear, as many as ten anthers were required because various stages were often represented in the same bud.

Buds in good condition were split. One half was stripped of sepals, fixed at once in Navashin's fluid (Karpechenko formula), dehydrated through a long series of alcohols, and later imbedded. The other half was used to make permanent smears, after the method of TAYLOR (**26**). For smear preparations fixation in Navashin's fluid also proved most satisfactory.

The schedule used in staining smears was essentially SMITH's (**23**) modification of the Gram stain. However, crystal violet in 0.5 of 1 per cent solution and the iodine-potassium-iodide similarly diluted proved easier to control. The picric acid wash was a saturated solution diluted four times. This method of staining gave a clear yellow cytoplasm and purple chromosomes.

Root tips were fixed in Allen's modification of Bouin's fluid, Zenker's fluid, modified Gilson's fluid, chrom-acetic, Navashin's fluid, and strong Flemming diluted about one-half. The last was most efficient. Mitoses went on rapidly from 10 A.M. until early afternoon. Tips from aerial roots were cut from the plant and allowed to stand for a few minutes in distilled water. This softened the mucilage that covered the root so that it could be largely removed by wiping with cotton. When the root tips were no longer slippery, they were slit up one side to aid penetration and cut back to within 5 mm. of the tip.

Iron-alum haematoxylin proved to be a more valuable stain for root tips than crystal violet, particularly when picric acid was used as a destaining agent (**29**).

Microsporogenesis in Echinocereus papillosus

Echinocereus papillosus Linke, selected for a study of meiosis, is native to southern Texas and is found over a large area. Both sec-

tioned and smeared material were used. Prophase smears were found to be of little value. The chromosomes in the Cactaceae are minute and not favorable for critical studies on chromosome structure.

The resting nucleus of the microsporocyte is less than 10 μ in thickness (fig. 1)[2] and presents the reticulum of fine threads characteristic of this stage. There is usually only one nucleolus. This body stains deeply during early prophase and does not begin to lose its chromaticity until late pachynema. It does not disappear until just before metaphase I, when it fades gradually but does not change from its spherical form.

With the beginning of prophase, the stage of leptonema (fig. 2) becomes clear. There is a definite thickening of the threads, and at the same time they show a tendency to run parallel to one another, which is sharply defined just before the stage of synizesis (fig. 3). At this time the nucleus begins to increase in size, and with this enlargement is possibly associated the condition of apparent contraction of the chromatic material, which may be due at least in part to imperfect fixation. The late stages of leptonema show a definite pairing of threads, which become closely associated. As has been pointed out by others, this association of threads (fig. 4) in a parasynaptic relation does not take place simultaneously all along the threads, but begins at random points.

With the increase in size of the nucleus, a marked thickening of the threads takes place which carries the nucleus into pachynema (figs. 5, 6). The parallel threads now become closely associated and apparently twisted. This is accompanied by a marked shortening of the elements. Coincident with this stage is the appearance of free ends at the periphery of the nucleus (fig. 7). In this species the tetrad structure of the chromosome is not obvious at metaphase of the first division. The lengthwise splitting of each chromosome may take place much earlier, but it is not apparent until early anaphase of the first division.

The close approximation of the synaptic mates continues through late pachynema, with further condensation of the threads (fig. 8). Finally during early diakinesis an apparent loosening of the association occurs which frees the members of the pairs except at one or

[2] See plate I, opposite page 20.

both ends (figs. 9, 10). The pairs of chromosomes condense still further and assume the forms characteristic of diakinesis (fig. 11). At this time the nucleus begins to decrease in size until it is again approximately 10 μ in thickness (fig. 12).

The chromosomes as they appear at metaphase I (fig. 13) are oval or round in outline, with an average width of from 1.7 to 2.0 μ and a length of from 2.0 to 2.5 μ. These dimensions represent an average for all the species studied. Throughout the tribe there is little difference in chromosome size at meiosis. The spindle establishes itself quickly following diakinesis, and the chromosomes become arranged at the plate. As seen from the pole, they are usually placed in a circle of eight with three in the center. The spindle fiber attachment for most of the chromosomes is median, but in some it is terminal. The chromosomes at anaphase I barely reach the poles before they show that they are split (fig. 14).

During interkinesis the sister chromatids tend to separate and to elongate (fig. 15), but again condense in preparation for the second division. The nucleolus which appeared at the beginning of interkinesis fades away soon after the disappearance of the nuclear membrane (fig. 16). Metaphase II is shown in figure 17 and anaphase in figure 18. The four daughter nuclei, after a long telophase, return to a resting condition (fig. 19). Furrows develop at the periphery of the sporocyte and proceeding inward apparently cut out the microspores (fig. 20).

In this species the pollen is almost wholly fertile, and there is no evidence of meiotic irregularity. The absence of lagging and nondisjunction indicates that the material is not of hybrid origin.

A matter of great interest was the appearance in this species, as well as in the majority of others studied, of certain bodies in the cytoplasm whose function and origin are obscure. They were first noticed in the material during interkinesis. They may be seen in early prophase and through diakinesis as two spherical or oval bodies at opposite ends of the cell (fig. 5). During metaphase I they arrange themselves on either side of the spindle. At anaphase I and through interkinesis and prophase II they lie close to the periphery of the cell, where they are conspicuous. At any time after metaphase I, four such bodies may be present. During interkinesis, two of the four

bodies are generally much more prominent (fig. 15) and remain so through prophase II (fig. 16). As the nuclei pass into metaphase II, the bodies become much less evident but are visible again in second anaphase (figs. 17, 18). When telophase II is initiated the four bodies come to lie one at the side of each nucleus, and shortly there are two by each nucleus. They fade away at this point.

The only reference to such structures noted in the literature is that of CASTETTER (5) in his paper on *Melilotus*. He figures similar bodies on either side of the metaphase I plate. In his discussion he refers to them as centrosome-like bodies, but states that he is unable to come to any conclusion as to their history or function.

The only further information which this study can offer is the reaction which these structures give to various stains and reagents. In general their staining reactions follow that of the chromatic material. It is necessary that the stain be left rather heavily in the nucleus, if these bodies are to show clearly. In prophase, when the nucleolus was prominent, they were more obscure. They are sharpest just before the formation of the metaphase II plate.

The bodies are not destroyed by fluids containing chromic acid, formaldehyde, or acetic acid. They also occur when material is fixed in a modification of Bouin's fluid in which anthroquinone replaces picric acid (1). They are partially or completely destroyed by a diluted Gilson's fluid. They show fairly well in sections or smears when stained with iron-alum haematoxylin. Crystal violet and safranin both bring them out brilliantly; the best results in the use of both stains are obtained when a counter wash of picric acid is employed. Alizarin will also stain them, but not sharply.

Chromosome configurations in other species

The results of the examinations of other species are given in the order in which the species are arranged by BRITTON and ROSE. The stages of meiosis best adapted for studies of chromosome configuration are diakinesis and late interkinesis. In root tips the metaphase plate is most useful.

The chromosomes in meiosis are compact and so small that peculiarities of size and form are difficult to recognize. Usually one pair is larger than the others, two to four pairs are medium sized,

and the remaining pairs consist of very small and almost spherical chromosomes.

Drawings of the chromosomes in the root tips were made from cells near the outermost layer, since these cells are larger and likely to be better fixed than those in the interior. They were never taken from further in than the sixth row and usually from the second or third.

In the following list, the numbers placed after the species names are accession numbers at the Botanical Garden of the University of Michigan.

1. *Wilcoxia poselgeri* (Lemaire) Br. & R. (15275): Diploid, twenty-two chromosomes; eleven pairs at metaphase I of meiosis (fig. 21); chromosomes all about the same size, ten pairs with median spindle fiber attachment, one with terminal attachment.

2. *Nyctocereus serpentinus* (Lagasca & Rod.) Br. & R. (13918): Diploid, twenty-two chromosomes; eleven pairs shown at metaphase I of meiosis (fig. 22); spindle fiber attachments seem to be terminal or slightly subterminal.

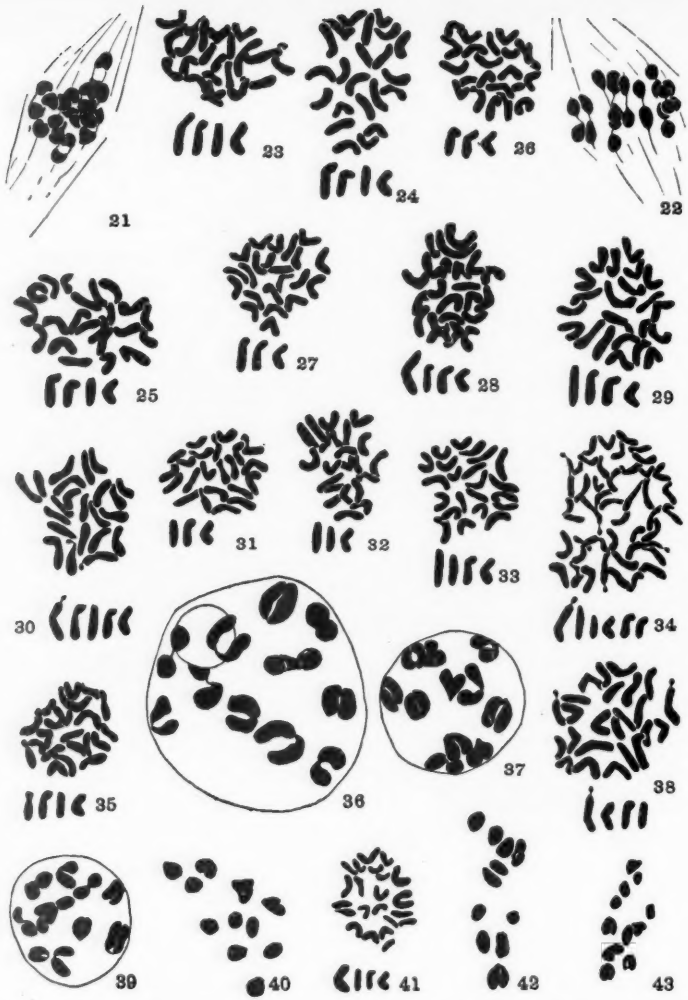
3. *Acanthocereus pentagonus* (Linnaeus) Br. & R. (15277): Diploid, twenty-two chromosomes; root tip metaphase (fig. 23); two chromosomes large with submedian attachments, four subterminal, six terminal, ten median or nearly median.

4. *Hylocereus guatemalensis* (Eichlam) Br. & R. (13941): Diploid, twenty-two; four groups of chromosomes in root tip metaphase (fig. 24); two largest chromosomes have spindle fiber attachments subterminal, two submedian, six terminal, twelve median varying considerably in size.

5. *Hylocereus purpusii* (Weingart) Br. & R. (13900): Diploid, twenty-two; root tip metaphase (fig. 25); chromosomes fall into the same groups as do those of the preceding species, to which it is closely related.

6. *Hylocereus undatus* (Haworth) Br. & R. (1526): Diploid, twenty-two; root tip metaphase (fig. 26); four chromosomes with subterminal attachments, six with submedian, twelve median; chromosomes more uniform in size and shape than those in the preceding two species.

7. *Hylocereus cubensis* Br. & R. (13997): Diploid, twenty-two; root tip metaphase (fig. 27); since this species is closely related to



FIGS. 21–43.—Fig. 21, *Wilcoxia poselgeri*, metaphase I. Fig. 22, *Nyctocereus serpentinus*, metaphase I. Root tip metaphase of: fig. 23, *Acanthocereus pentagonus;* fig. 24, *Hylocereus guatemalensis;* fig. 25, *Hylocereus purpusii;* fig. 26, *H. undatus;* fig. 27, *H. cubensis;* fig. 28, *H. monacanthus;* fig. 29, *H. triangularis;* fig. 30, *Selenicereus hondurensis;* fig. 31, S. *pteranthus;* fig. 32, *S. kunthianus;* fig. 33, *S. spinulosus;* fig. 34, *Mediocactus coccineus* (with 44 chromosomes); fig. 35, *Werkleocereus glaber*. Fig. 36, *Echinocereus angusticeps*, diakinesis. Fig. 37, *E. pentalophus*, diakinesis. Fig. 38, *E. blanckii*, root tip metaphase, diploid 24 chromosomes. Fig. 39, *E. reichenbachii*, diakinesis. Fig. 40, *E. fitchii*, prophase II. Fig. 41, *Lophophora williamsii*, root tip metaphase. Fig. 42, *Hamatocactus setispinus* var. *hamatus*, prophase II. Fig. 43, *H. setispinus* var. *setaceous*, prophase II.

H. undatus, it would be expected to have similar chromosome complements, which is the case.

8. *Hylocereus monacanthus* (Lemaire) Br. & R. (14067): Diploid, twenty-two; root tip metaphase (fig. 28) shows chromosomes of this species distinctly different from others in the genus; two chromosomes half again as long as the next largest appear to have median attachments, two subterminal, eight submedian, ten median.

9. *Hylocereus triangularis* (Linnaeus) Br. & R. (1569): Diploid, twenty-two; root tip metaphase (fig. 29); two chromosomes longer than the rest, with terminal attachments, four subterminal, six submedian, ten median.

10. *Selenicereus hondurensis* (Schumann) Br. & R. (14688): Diploid, twenty-two; root tip metaphase (fig. 30); two chromosomes of the set with spindle fiber attachments submedian have satellites on the shorter arm, four have attachments subterminal, ten terminal, four submedian, two median.

11. *Selenicereus pteranthus* (Linke & Otto) Br. & R. (1547): Diploid, twenty-two; root tip metaphase (fig. 31) presents chromosomes similar in size and form; six short with terminal attachments, six subterminal, ten median.

12. *Selenicereus kunthianus* (Otto) Br. & R. (1360): Diploid, twenty-two; root tip metaphase (fig. 32); eight chromosomes with terminal attachments, remainder with median or near median attachments and similar in size.

13. *Selenicereus spinulosus* (DeCandolle) Br. & R. (13697): Diploid, twenty-two; root tip metaphase (fig. 33); six chromosomes terminally attached, four have subterminal attachments, remaining twelve attachments are all nearly median.

14. *Mediocactus coccineus* (Salm-Dyck) Br. & R. (7520): Diploid, forty-four chromosomes; root tip metaphase (fig. 34); four chromosomes, among the largest in the complement, have subterminal attachments and appear to bear satellites on the longer arm, four other long chromosomes with terminal attachments, four short chromosomes with terminal attachments, sixteen with median attachments, eight subterminal and eight submedian. This species is the only tetraploid so far known in this subtribe. The material was obtained from a plant in the greenhouses of the Botanical Garden of the Uni-

versity. It has not flowered since the writer began work on the material, so there has been no opportunity for studies of meiosis. The cells of the root are noticeably larger than those of related diploid species, but the chromosomes are smaller and more slender than those found in normal diploid cells.

15. *Werkleocereus glaber* (Eichlam) Br. & R. (7553): Diploid, twenty-two; root tip metaphase (fig. 35); two chromosomes with subterminal attachments, two with attachments more markedly subterminal, two with terminal attachments, sixteen median or nearly so.

16. *Echinocereus papillosus* Linke (15255): Diploid, twenty-two; diakinesis figure shows eleven pairs of chromosomes (fig. 11).

17. *Echinocereus angusticeps* Clover (15261): Diploid, twenty-two; eleven pairs are evident at diakinesis (fig. 36); in about 20 per cent of the cells examined there is non-disjunction at metaphase I, ten and twelve chromosomes passing to either pole. This species is most closely allied to *E. papillosus* and is possibly derived from it. Morphologically it resembles that species but is much smaller. Its habitat is different from that of *E. papillosus* in that it grows in sandy loam in open woods, while the other species is found on gravel or limestone hills. The two species are found within a few miles of each other, but their ranges apparently do not overlap.

18. *Echinocereus pentalophus* (DeCandolle) Rümpler (15253): Diploid, twenty-two; eleven pairs evident at diakinesis (fig. 37); this species differs from the two in the same genus previously discussed in being a slender procumbent plant whereas the others are erect. The spines are borne on ridges rather than on papillae, and the flowers are rich mallow purple with white centers as compared with the pale yellow and maroon red of the foregoing two species.

19. *Echinocereus blanckii* (Poselger) Palmer (15268): Diploid, twenty-four; root tip metaphase (fig. 38); two chromosomes having the spindle fiber attachments subterminal bear satellites on the longer arm, twelve chromosomes have median attachments, four subterminal, six terminal. This species is morphologically different from the preceding species in the increase in the number of ribs, the presence of definite papillae bearing the spine areoles, and the appearance of flowers which are entirely mallow purple. Cytologically this spe-

cies is interesting because the haploid number of chromosomes is twelve. In cultivation the plants bloom poorly, and a large percentage of the buds abort. Such buds as mature do not show complete pollen fertility. No fruit has been reported for this species by BRITTON and ROSE. All evidence yet available suggests that *E. blanckii* came out of *E. pentalophus* by the addition of an extra chromosome through non-disjunction.

20. *Echinocereus reichenbachii* (Terschek) Haage Jr. (15262): Diploid, twenty-two; diakinesis figure presents the usual eleven pairs of chromosomes (fig. 39); the plant is one of wide range and adaptability.

21. *Echinocereus fitchii* Br. & R. (15267): Diploid, twenty-two; haploid number of eleven shown at metaphase II (fig. 40).

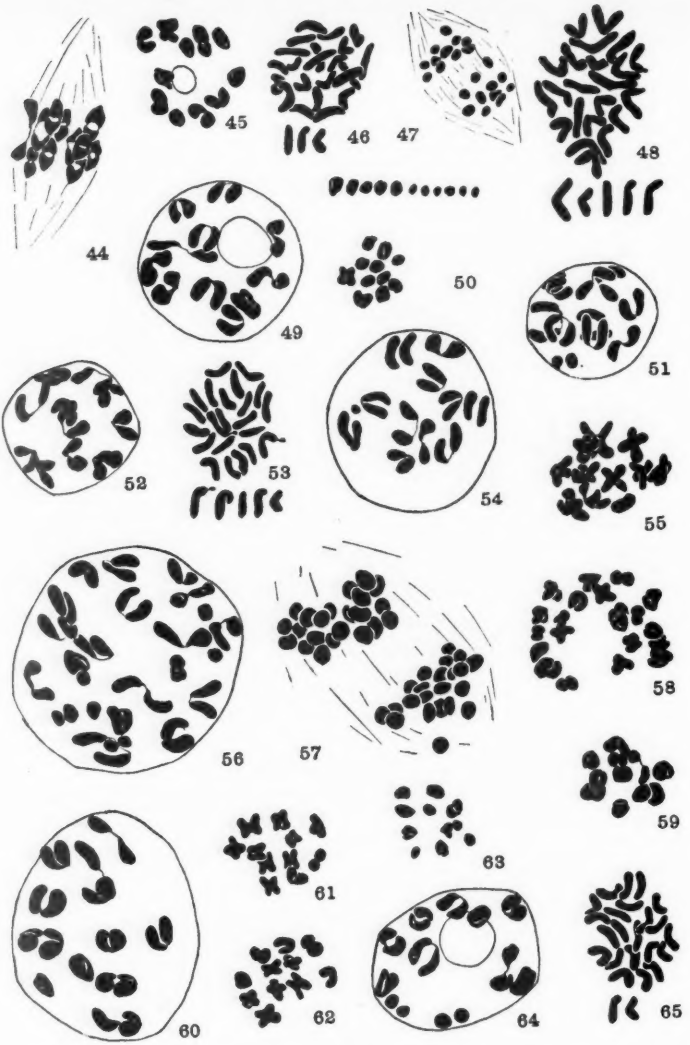
22. *Echinocereus enneacanthus* Engelmann (15256): Diploid, twenty-two (?); chromosomes were studied in root tips, all found to be nearly the same size.

23. *Lophophora williamsii* (Lemaire) Coulter (15269): Diploid, twenty-two; root tip metaphase interesting in that all but one pair of chromosomes are very small (fig. 41); two largest chromosomes have median attachments, two subterminal, four terminal, and fourteen median or nearly so.

24. *Hamatocactus setispinus* (Engelmann) Br. & R. (15295, 15297): Diploid, twenty-two; prophase II figures show eleven split chromosomes (figs. 42, 43); in the species there are two distinct forms which have been investigated. Morphological variations involving number of ribs, presence or absence of papillae on the ribs, and number and shape of the spines are found. ENGELMANN went so far as to give varietal names to the forms, but these are not recognized by BRITTON and ROSE. Observations in the field have led the writer to believe that these types should be recognized as distinct. Both have a haploid number of eleven, but there is a marked difference in chromosome size.

25. *Echinofossulocactus grandicornis* (Lemaire) Br. & R. (16142): Diploid, twenty-two (?); root tip material too scant to permit positive statement.

26. *Ferocactus hamatacanthus* (Muhlenpfordt) Br. & R. (15271):



FIGS. 44–65.—Fig. 44, *Ferocactus hamatacanthus*, metaphase I. Fig. 45, *Homalocephala texensis*, prophase II. Fig. 46, *Astrophytum asterias*, root tip metaphase. Fig. 47, *Thelocactus bicolor*, anaphase II. Fig. 48, *Coryphantha runyonii*, diakinesis. Fig. 49, *Escobaria runyonii*, diakinesis. Fig. 50, *Dolicothele longimamma*, prophase II. Fig. 51, *Neomammillaria heyderi*, diakinesis. Fig. 52, *N. hemisphaerica*, diakinesis. Fig. 53, *N. magnimamma*, root tip metaphase. Fig. 54, *N. chinocephala*, diakinesis. Fig. 55, *N. aureiceps*, prophase II, characteristic H shaped split chromosomes. Fig. 56, *N. multiceps*, tetraploid, 44 chromosomes showing pairing. Fig. 57, *N. multiceps*, anaphase I. Fig. 58, *N. multiceps*, prophase II. Fig. 59, *N. decipiens*, prophase II. Fig. 60, *N. longicoma*, diakinesis. Fig. 61, *N. tenampensis*, prophase II. Fig. 62, *N. minuta*, prophase II. Fig. 63, *Epiphyllum strictum*, anaphase I. Fig. 64, *E. ackermannii*, diakinesis Fig. 65, *Rhipsalis mesembrianthemoides*, root tip metaphase.

Diploid, twenty-two; figure of metaphase I shows eleven pairs of chromosomes (fig. 44).

27. *Homalocephala texensis* (Hopffer) Br. & R. (15272): Diploid, twenty-two; prophase II shows haploid number of eleven (fig. 45); this species is representative of a small genus closely related to *Echinocactus*.

28. *Astrophytum asterias* (Zuccarini) Lemaire (15274): Diploid, twenty-two; root tip metaphase (fig. 46); ten chromosomes straight with no evident constrictions, suggesting terminal attachments; two attachments subterminal, and ten with attachments median.

29. *Thelocactus bicolor* (Galeotti) Br. & R. (15294): Diploid, twenty-two; eleven chromosomes shown in anaphase II (fig. 47).

30. *Coryphantha runyonii* Br. & R. (15270): Diploid, twenty-two; root tip metaphase, with chromosomes distinctly larger than those in preceding genera (fig. 48); fourteen chromosomes with median spindle fiber attachments, two with terminal attachments, four subterminal, and two submedian; three pairs of chromosomes were pointed at the tips, suggesting the presence of satellites.

31. *Escobaria runyonii* Br. & R. (15303): Diploid, twenty-two; eleven pairs of chromosomes clear at diakinesis (fig. 49).

32. *Dolicothele longimamma* (DeCandolle) Br. & R. (1540): Diploid, twenty-two; the usual eleven split chromosomes at metaphase II (fig. 50); chromosomes show wide range in size, one pair large, four medium, six small.

33. *Neomammillaria heyderi* (Muhlenpfordt) Br. & R. (15286): Diploid, twenty-two; diakinesis shows eleven pairs of chromosomes (fig. 51).

34. *Neomammillaria hemisphaerica* (Engelmann) Br. & R. (15287): Diploid, twenty-two; diakinesis, eleven pairs of chromosomes (fig. 52); this species is closely related to *N. heyderi*.

35. *Neomammillaria magnimamma* (Haworth) Br. & R. (16164): Diploid, twenty-two; root tip metaphase (fig. 53); two chromosomes have attachments submedian and appear to carry a satellite.

36. *Neomammillaria compressa* (DeCandolle) Br. & R. (1520): Diploid, forty-four; pollen mother cells at interkinesis show twenty-two pairs of chromosomes; chromosomes give evidence of lagging and non-disjunction; material in greenhouse failed to set fruit.

37. *Neomammillaria chinocephala* (Purpus) Br. & R. (16172): Diploid, twenty-two; eleven pairs of chromosomes at diakinesis (fig. 54); a fragment appeared at diakinesis in addition to the normal set; material scant, complete history not available.

38. *Neomammillaria aureiceps* (Lemaire) Br. & R. (1541): Diploid, twenty-two; prophase II shows eleven split chromosomes in characteristic H shapes (fig. 55).

39. *Neomammillaria multiceps* (Salm-Dyck) Br. & R. (1538 and 15201): Diploid, forty-four; diakinesis shows twenty-two pairs of chromosomes (fig. 56). Two distinct forms of this species are under cultivation at the University of Michigan Botanical Garden. The larger of them, which is fully three times the size of the other and which has certain definite peculiarities of spine color and arrangement, was collected in the lower Rio Grande Valley and was also present in the collection from the Missouri Botanical Garden. The smaller form was also collected in the lower Rio Grande Valley. Both of them are tetraploid with identical behavior in meiosis. Diakinesis (fig. 56) shows a close association of the chromosomes in pairs with no evidence of groupings in fours. In several hundred cells examined, only one showed four homologues together. Separation after metaphase I is prompt and complete (fig. 57). The split preparatory to second division is not so well defined in early anaphase I as it is in normal diploids. The prophase of the second division is entirely normal. The chromosomes are so small that no distinguishing characteristics can be noted in either set.

40. *Neomammillaria decipiens* (Scheidw.) Br. & R. (7546): Diploid, twenty-two; metaphase II shows eleven split chromosomes (fig. 59).

41. *Neomammillaria longicoma* Br. & R. (16161): Diploid, twenty-two; eleven pairs of chromosomes at diakinesis (fig. 60).

42. *Neomammillaria tenampensis* Br. & R. (16151): Diploid, twenty-two; prophase II, eleven split chromosomes (fig. 61); interesting because of similarity to *N. aureiceps*.

43. *Neomammillaria minuta* Bartlett unpub. man. (13756): Diploid, twenty-two; prophase II shows eleven split chromosomes (fig. 62); pollen mother cells smaller than average.

44. *Epiphyllum strictum* (Lemaire) Br. & R. (13901): Diploid,

twenty-two; metaphase II (fig. 63); chromosomes much smaller than average.

45. *Epiphyllum ackermanni* Haworth (12737): Diploid, twenty-two; diakinesis, eleven pairs of chromosomes (fig. 64); the form is interesting because it is generally supposed to be a hybrid.

46. *Rhipsalis mesembrianthemoides* Haworth (7539): Diploid, twenty-two; root tip metaphase (fig. 65); chromosome complement very simple; short chromosomes with either terminal or median attachments.

Discussion

In the past decade much has been written on the relation of chromosome complements to taxonomy. TISCHLER (**27**) discusses the conditions which have been reported and classifies them as follows:

1. The *Pinus* type, in which all species studied in a given family have a uniform chromosome count.

2. The *Chrysanthemum* type, in which species within a genus present a straightforward polyploid series.

3. The *Carex* type, genera with species presenting chromosome numbers or series of numbers apparently unrelated.

4. The *Antirrhinum* type, in which each genus has a basic number which is strictly followed but in which each genus is separated from those closely related to it by the difference of one chromosome.

JÖRGENSEN (**18**) arranges types to include (a) genera in which all species studied have the same number; (b) genera with aneuploid numbers; (c) genera with numbers in multiple relations. It has become increasingly evident as more material is handled that many exceptions will be found to such classifications.

The cacti seem likely to fall chiefly into the first group of both JÖRGENSEN and TISCHLER, in that all of the twenty-one genera here considered contain the basic number of eleven, as do those reported elsewhere. One genus, *Mediocactus*, is represented so far only by a tetraploid, but the evidence indicates that in this case eleven is also the basic number. At the same time there is evidence (**24**) of a well developed polyploid line in *Opuntia*. SUGIURA (**25**) reports a haploid count of twelve for two species in two genera. STOCKWELL records a haploid number of nine for *Neomammillaria applanata* (Engelmann) Br. & R. The writer finds one aneuploid (*Echinocereus*

blanckii) and three tetraploids (*Neomammillaria multiceps*, *N. compressa*, *Mediocactus coccineus*). Briefly, these constitute the exceptions to the general classification.

As has been suggested, the tribe Opuntieae may be considered more primitive than the Cereeae. It is interesting to note in this connection that so far no basic number other than eleven has been found in the former tribe, and that the only aneuploids reported fall in the Cereeae.

The Cereeae, with which this paper is primarily concerned, contains several scattered tetraploids as well as certain aneuploids. The information available does not permit more than conjecture as to their origin, but certain observations can be made concerning their behavior and position.

Mediocactus coccineus, which is the only tetraploid reported from the subtribe Hylocereaneae, is a relatively primitive type. It is a climbing form found in Brazil and Argentina. There are four chromosomes which are alike in shape and size and which appear to bear satellites. This suggests that the plant is a possible autotetraploid (fig. 34).

Neomammillaria multiceps is a tetraploid (fig. 56) in which the chromosomes at meiosis are so nearly alike that it has not been possible to differentiate them. As has been stated, the chromosomes are present in pairs at diakinesis. Only rarely do they appear in fours. The view has been generally held that the chromosomes of autotetraploids associate in fours with a fair degree of regularity, and such behavior has been reported in *Aucuba* (**21**), *Datura* (**2**), *Hyacinthus* (**8**), *Solanum* (**19, 20**), *Primula* (**9**), and *Prunus* (**7**). On the other hand, DAVIS (**10**), in describing an *Oenothera* known to be autotetraploid, reports that the chromosomes do not form groups of four but are in pairs at diakinesis. It has been suggested that autotetraploid plants may in time undergo sufficient stabilization through differentiation of homologous chromosomes so that they become digenomic. The behavior suggests that this may have occurred here, since there is only one species to which *Neomammillaria multiceps* is obviously closely allied (*N. prolifera*), and there is no evident way by which it could have arisen through hybridization. Dr. ELZADA CLOVER of the University of Michigan Botanical Garden has secured

germination of 90 per cent or more from seeds of different plants of this species. The range of *N. multiceps* is not great. It is found from the coast to approximately 100 miles up the Rio Grande River valley and a short distance south into Mexico.

The third tetraploid, *N. compressa*, shows pairing to a large extent at diakinesis, but metaphase I is often disturbed and a few univalents occur. It flowers freely but has not set fruit in the greenhouses of the University. This species is common in Mexico, and has a number of well recognized varieties which suggest a series of forms similar to that reported for *Opuntia polyacantha* (24).

The only aneuploid discovered in the present material is *Echinocereus blanckii*, with a haploid number of twelve. As was pointed out, this species is very similar to *E. pentalophus*, except that (a) the flower is a uniform mallow purple, lacking the white center of *E. pentalophus;* (b) the plant is characteristically 5–6 ribbed rather than 4–5; (c) the ribs have more prominent papillae; (d) the plants have heavy tuberous roots which are not found in the other species. Both species are found in the region of the lower Rio Grande River valley, although *E. blanckii* is distinctly more limited in range. It is possible that this species arose from *E. pentalophus* through the addition of one chromosome by non-disjunction. There are many cases of aneuploidy on record, among them members of *Primula* (4), *Viola* (6), *Crepis* (22), *Antirrhinum* (13), *Lactuca* (15), and *Carex* (11, 12). Aneuploidy may be expected in a group as large as the Cactaceae.

Satellites have been reported in the Cactaceae by JOHANSEN (17) and STOCKWELL (24). The writer found satellites in *Selenicereus hondurensis*, *Mediocactus coccineus*, *Echinocereus blanckii*, and *Neomammillaria magnimamma*.

Primary constrictions appear with varying degrees of clarity in somatic chromosomes. Usually they are clearly indicated by a bend in the chromosome. An attempt has been made to group the chromosomes on the basis of primary constrictions. The random sampling here reported is not sufficient to present an accurate picture of chromosome morphology in the Cereeae, but it does suggest some possibilities of relationships in chromosome configuration.

The facts that the basic number eleven is so consistently present

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in all of the genera studied, and that it is the haploid number for the majority, suggest strongly that evolution in this family has been accomplished largely through gene mutations. The two factors which appear to rank next in importance in the development of the family are polyploidy and hybridization.

From the available information, the Cereeae do not seem to show as strong an inclination toward polyploidy as do the Opuntieae. Such polyploids as do occur in the Cereeae may arise through somatic doubling. There appears to be a stronger tendency in this tribe to develop aneuploids, possibly owing to the fact that the genera are more divergent than in the Opuntieae.

The evidence seems to show that the family is still relatively young, in that it is known to be giving rise to numerous new types.

Summary

1. The cytological work on record is briefly surveyed. There are reports on only nineteen species. Work has been confined to members of the tribe Cereeae, which is the most highly evolved tribe in the family and contains the greatest number of genera.

2. The chromosome numbers are given for forty-six species, including two forms and one hybrid. The basic number, eleven, is present in every genus here reported, and is the haploid number in the majority of species.

3. Three tetraploids are recorded and discussed as to origin. Of these, *Mediocactus coccineus* is one of the simpler Cereeae and the others, *Neomammillaria compressa* and *N. multiceps*, are both members of the largest genus in the tribe.

4. One aneuploid species, *Echinocereus blanckii*, is described. It is suggested that it arose out of *E. pentalophus* by reduplication of one chromosome through non-disjunction.

5. Meiosis of *Echinocereus papillosus* was studied. The behavior is reported as entirely normal. The prophase threads associate by parasynapsis.

6. Certain bodies in the cytoplasm are described as they occur in *E. papillosus* and a majority of the other species studied. They can commonly be seen in early pachynema as two oval structures lying at either end of the cell. Their staining reactions become more in-

tense as prophase advances and is greatest just before telophase I. At this time or shortly after, two other such bodies appear which come to lie between the two nuclei. At the end of second division there is one body beside each nucleus. Often eight bodies are found at the end of telophase II.

7. The relation of the chromosome numbers to taxonomy is discussed, and it is pointed out that the rather simple chromosome situation is in keeping with the morphological evidence that the tribe Cereeae is young.

The writer expresses to the Botanical Garden of the University of Michigan her gratitude for assistance which made this study possible. It is with pleasure that I acknowledge indebtedness to Professor BRADLEY M. DAVIS for his interest in the progress of the work and his helpfulness in the preparation of this paper. Thanks are also due Dr. ELZADA CLOVER for the determination of material and the Shiner Cactus Nursery, Laredo, Texas and the Rio Grande Valley Cactus Garden, Edinburg, Texas for their cooperation in making available certain rare material.

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UNIVERSITY OF MICHIGAN

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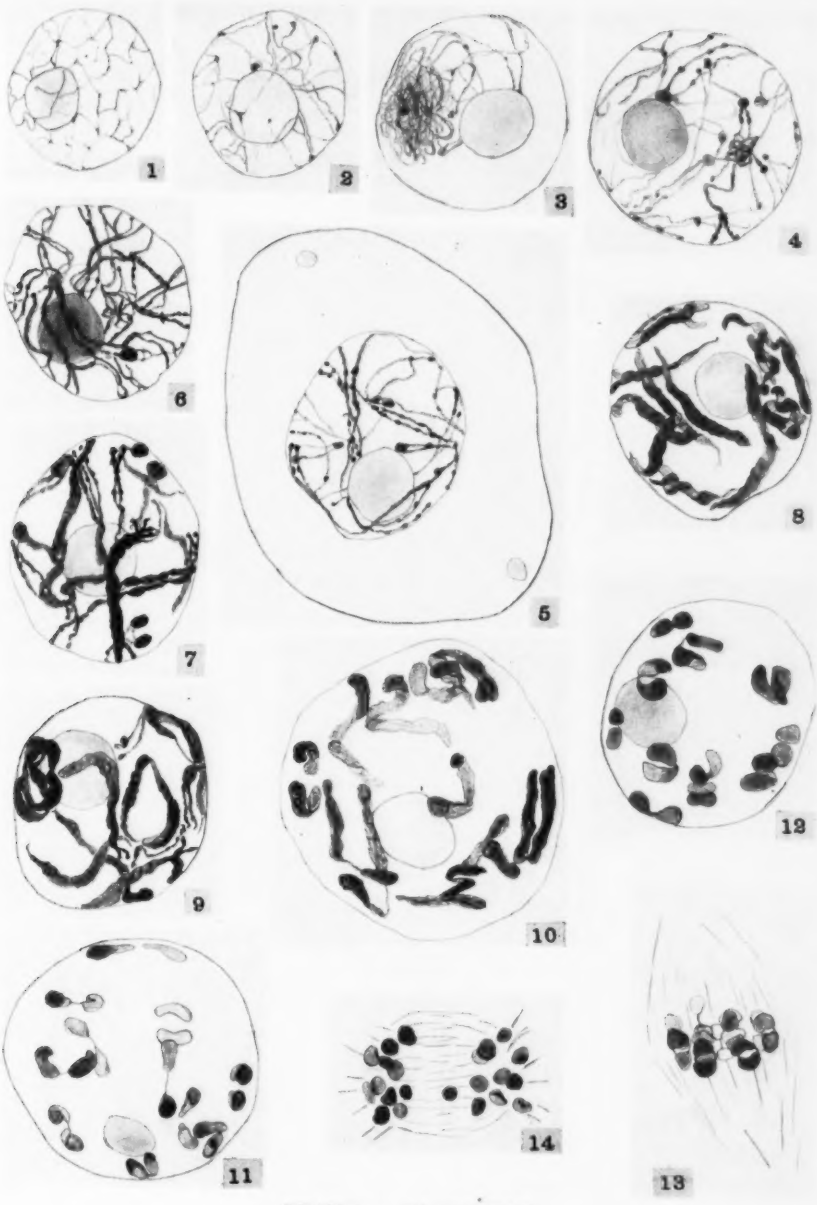
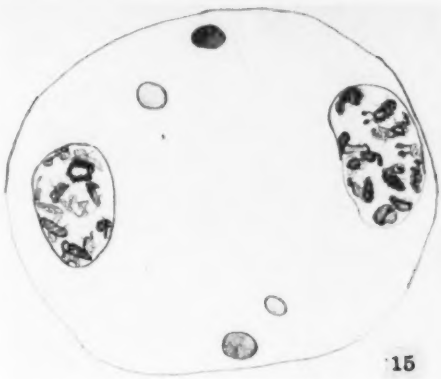
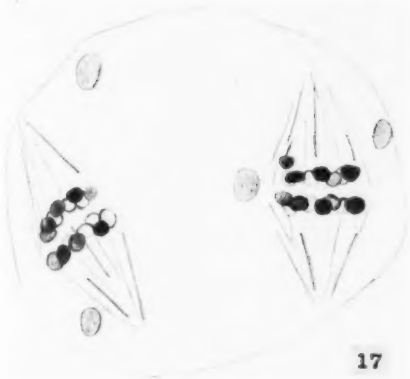


BEARD on CACTACEAE





BEARD on CACTACEAE

EXPLANATION OF PLATES I, II

All figures were drawn with the aid of a camera lucida under Zeiss apochromatic objective 1.5 (120×) in combination with the ocular K 20×. Measurements were made with an eyepiece micrometer in the Zeiss K 10× ocular.

Echinocereus papillosus Linke

PLATE I

FIG. 1.—Resting nucleus from archesporium.
FIG. 2.—Late leptonema.
FIG. 3.—Synizesis.
FIG. 4.—Early pachynema, parallel threads evident.
FIG. 5.—Middle pachynema, appearance of cytoplasmic bodies.
FIG. 6.—Zygonema conspicuous.
FIG. 7.—Late pachynema, appearance of four closely associated threads.
FIG. 8.—Chromosome segments defined.
FIG. 9.—Diakinesis, two chromonemata visible.
FIG. 10.—Diakinesis.
FIG. 11.—Late diakinesis.
FIG. 12.—Diakinesis, condensation practically complete.
FIG. 13.—Metaphase I.
FIG. 14.—Anaphase I, chromosomes beginning to show split preparatory to second division.

PLATE II

FIG. 15.—Interkinesis, four bodies present in cytoplasm.
FIG. 16.—Prophase II, four bodies in cytoplasm, nucleolus also present.
FIG. 17.—Metaphase II.
FIG. 18.—Anaphase II.
FIG. 19.—Telophase II, eight bodies in cytoplasm.
FIG. 20.—Tetrad showing cleavage by furrowing.

MECHANISM AND QUANTITATIVE APPLICATION OF THE PEA TEST

J. VAN OVERBEEK AND F. W. WENT

(WITH EIGHT FIGURES)

Introduction

Some years ago WENT (9) published a new method for determining the growth hormone (auxin) concentration of solutions, the so-called pea test. If the rapidly elongating region of an etiolated pea stem is split lengthwise and placed in water, the two halves will bend outward owing to tissue tension. If the split stems are placed in solutions containing auxin, however, the growing zones will bend inward, assuming an S or crescent outline. The inward curvature is proportional to the log of the auxin concentration. The advantages of the pea test over the standard *Avena* test can be summarized as follows:

1. Large range of concentrations in which the effect is a function of the concentration.

2. Humidity need not be constant.

3. Curvature reaches a maximum in the course of 4 to 12 hours and does not change thereafter, so that the test can be measured at any time between 12 and 60 hours after the beginning of the experiment.

4. No lack of sensitivity, if occasionally exposed to small amounts of white light.

The pea test, however, has certain disadvantages as compared with the *Avena* test, since (*a*) it requires about 20 cc. of the active solution whereas the *Avena* test can be carried out with less than 1 per cent of this amount; (*b*) it is less specific in its reaction, so that a number of substances which in the *Avena* test give only a very slight reaction, and then only in a very high concentration, are active in the pea test; (*c*) it is not so quantitative as the *Avena* test.

Point (*b*) has been turned into a distinct advantage. THIMANN (8) and HAAGEN-SMIT and WENT (4) have shown that substances,

hardly active in the *Avena* test but active in the pea test, would induce growth in length in short sections of *Avena* coleoptiles if suspended in a solution of those substances. They showed therefore that those particular substances were merely lacking the ability of being transported in the plant. Based on the more general response of the pea test, a completely new attack on the problem of the relation between chemical structure and physiological activity was made possible (**6**). Point (*c*) will be considered in the last part of this paper.

The only recent paper dealing with the mechanism of the pea test is one by JOST and REISS (**5**). In it they bring out the following points: 1. The pea test does not work so consistently as it should, so that often no curvatures at all are obtained. This must be attributed to unsuitable pea varieties and/or culture conditions. With Alaska peas there is some variation in sensitivity from day to day, but the test is always positive. 2. The tentative explanation given by WENT (**9**) is erroneous. It will be seen that we have come to the same conclusion. 3. *Taraxacum* flower stalks, like the pea stem, give auxin curvatures ("Wentreaktion") after being split lengthwise. Only when the stems are deficient in auxin they react and the reaction is caused by the increased growth of the outside.

Mechanism of pea test

Alaska peas were soaked in water for a few hours and then planted in moist sand. From then on the plants were grown in a physiological darkroom with controlled temperature and humidity (24° C. and 90 per cent respectively). All the experiments were performed in the darkroom under the same conditions. About one week after the seeds had been planted the pea seedlings were ready for use. From a number of uniform plants the terminal buds (about 5 mm. in length) were cut off. The upper 5 cm. of each remaining stump was used for the experiments. Such a piece consisted of an internode without the nodes. The stems were then marked into segments 1 mm. in length. This was done by placing them on the inked wires of a marking device[1] until at least half of the circumference showed thin parallel ink lines 1 mm. apart.

[1] The marking device consisted of a number of thin copper wires (0.005 of an inch in diameter) which were stretched parallel and 1 mm. apart on a brass frame. India

After the stems were marked, the apical 3 cm. was split lengthwise into two equal halves in such a way that both halves were plainly marked. The markings permitted an accurate measurement of the growth of the 1 mm. long segments at the wounded side (inside) and at the epidermis side (outside), even if the halved stems were strongly curved. Next the marked and split stems were put into petri dishes. Each dish contained 25 cc. solution and received three split stems (six halves). The stems were left in the solutions for about 18 hours, after which time they were removed from the darkroom and the length of the segments measured with an eye piece micrometer.

Pea test curvature caused by differential growth

The results of the experiments described in the previous section are summarized in table 1 and figure 1. The stems were put in tap water and in five different solutions of indole-3-acetic acid (hetero-auxin). The lowest concentration used contained 6.57 mg. of the hormone per 100,000 cc. of water. Such a concentration will give a curvature of about 6° in the standard *Avena* test. Table 1 gives the increase in length of the upper 25 mm. of the stems. In the table this 25 mm. has been subdivided into five zones, each having an initial length of 5 mm. The increase in length of these zones represents the sum of the increases of five segments of an original length of 1 mm. each. It may be seen that the upper 15 mm. of the stems represents the main growing region. Figure 1 shows that both the outside and the inside of the halved stems grow. In water (O on the abscissa) the inside grows somewhat more than the outside. This results in a negative traumatotropic curvature. In a concentration of 0.0657 mg. auxin per liter, the growth of both the inside and the outside is increased as compared with water. The inside grows slightly less than the outside. This difference in growth (curve o–i) causes the stems to curve positively. In still higher concentrations the growth of the outside increases with the concentration, whereas the growth of the inside decreases. This is probably not caused by a direct action of

ink was sparsely applied to the wires with a soft brush. An even marking over the entire stem was obtained by gently pressing it upon the wires by means of a flexible piece of rubber tubing. When the tubing was rotated the pea stem rolled over the wires and the ink marks showed clearly over at least half the surface of the stem.

the auxin on the growth of the inside, but is a mechanical effect due to the bending of the stem. For comparison, the growth of non-split stems is also given. Their growth increases with increasing concentration, and has in every instance a higher value than the outside of the split stems.

TABLE 1

GROWTH OF INSIDE AND OUTSIDE OF SPLIT AND OF NON-SPLIT PEA STEMS IN AUXIN SOLUTIONS. STEMS DIVIDED INTO FIVE SEGMENTS, EACH WITH INITIAL LENGTH OF 5 MM. FIGURES GIVE INCREASE IN LENGTH IN 0.1 MM. AND ARE AVERAGES OF 24 SPLIT STEMS AND 12 NON-SPLIT ONES. MEASURED 18 HOURS AFTER PLACING IN SOLUTIONS. EXPERIMENT NOS. 60714, 60715, 60718, AND 60721

SOLUTION	STEM	DISTANCE OF ZONE BELOW TOP				
		0–5 MM.	5–10 MM.	10–15 MM.	15–20 MM.	20–25 MM.
Water	Non-split	4.7±0.8	6.2±0.7	4.5±0.5	4.3±0.4	2.3±0.4
	Inside	2.9±0.4	3.5±0.3	2.6±0.2	2.9±0.2	2.0±0.3
	Outside	1.5±0.3	1.5±0.3	0.6±0.2	0.2±0.3	0.1±0.3
6.57 mg. heteroauxin per 100,000 cc.	Non-split	14.3±1.1	13.0±1.2	9.2±1.1	6.0±0.9	4.3±0.6
	Inside	9.7±0.7	7.7±0.7	5.6±0.5	4.3±0.4	2.6±0.3
	Outside	9.7±0.7	8.9±0.7	6.7±0.6	4.2±0.7	1.9±0.6
6.57 mg. heteroauxin per 40,000 cc.	Non-split	23.3±2.4	18.1±1.5	10.8±1.3	7.6±0.8	3.9±0.7
	Inside	9.5±0.9	6.0±0.6	5.4±0.5	4.7±0.4	3.3±0.4
	Outside	12.6±0.8	11.9±0.8	8.1±0.8	5.8±0.8	2.3±0.5
6.57 mg. heteroauxin per 20,000 cc.	Non-split	21.5±2.2	17.3±1.2	10.6±0.6	7.2±0.7	3.9±0.7
	Inside	8.5±1.1	6.5±0.8	5.9±0.4	4.1±0.3	3.2±0.4
	Outside	15.3±0.7	14.8±0.6	9.7±0.8	6.8±0.8	3.2±0.6
6.57 mg. heteroauxin per 10,000 cc.	Non-split	26.4±2.0	23.2±1.6	14.1±1.1	7.3±0.8	3.8±0.8
	Inside	5.9±0.8	4.0±0.5	5.1±0.4	4.8±0.3	3.4±0.5
	Outside	20.8±1.2	16.7±1.1	11.4±1.1	7.2±1.2	3.0±0.5
6.57 mg. heteroauxin per 4000 cc.	Non-split	26.9±2.0	22.9±1.9	16.4±1.6	8.5±1.3	5.0±1.0
	Inside	5.9±0.8	5.2±0.7	5.8±0.5	4.4±0.4	3.8±0.4
	Outside	24.7±1.5	23.0±1.1	16.4±0.8	7.0±0.8	3.0±0.6

After the completion of the experiments just described the article by JOST and REISS (5) appeared, in which these workers showed that upon immersion in an auxin solution the inside of a split *Taraxacum* stem grows less than its outside. Their results are in agreement with the facts presented here.

PREVENTION OF AUXIN UPTAKE THROUGH WOUNDED SURFACE

After it had been established that the inside grows less than the outside, the question rose as to the reason for this. The first experiment was as follows. Pea stems were split in the usual way and their bases inserted in vials of water. The apical split ends were in humid air under a bell jar in the darkroom. On the split end a lanolin-auxin paste was smeared in such a way that the paste covered either the

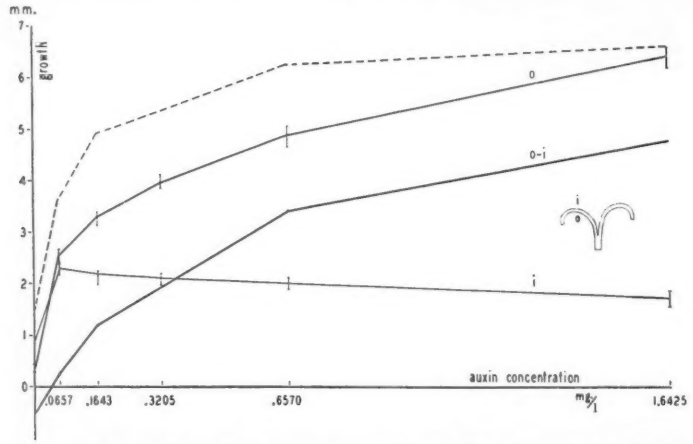


FIG. 1.—Increase in length of upper 15 mm. of inside (i) and outside (o) of halved pea stems and of non-split (broken line) pea stems in auxin solutions. Abscissa: concentration of indole-3-acetic acid in mg. per liter. Ordinate: increase in length in mm. during 18 hours. Small vertical lines indicate range of probable errors. Difference between growth of outside and inside indicated by o–i, which curve corresponds to actual "pea test curvature."

inside, the outside, or the apical ends of the halved stems. About 18 hours later the curvatures were measured. The results of this experiment are given in table 2. If no paste was applied the halves curved slightly negatively. In all cases application of the paste containing auxin resulted in a positive curvature. The largest curvature was obtained when the paste was applied to the outside; the smallest when the auxin was applied to the inside. A distinctly positive curvature was also obtained if the paste was applied to the apical ends of the halves. This would indicate that the response of the outside is greater than that of the inside.

In order to check this possibility, the following experiment was conducted. The epidermis of pea stems was removed and sections of stem 6.4 mm. long were cut. These were put in an auxin solution.

TABLE 2

CURVATURES OF SPLIT PEA STEMS WITH BASES INSERTED IN VIALS CONTAINING WATER AND SPLIT ENDS IN VERY HUMID AIR

EXPERIMENT NO.	PERCENTAGE CONCENTRATION OF AUXIN PASTE	PASTE ON OUTSIDE	NO. OF HALVES	PASTE ON INSIDE	NO. OF HALVES	PASTE ON TOP	NO. OF HALVES	NO PASTE APPLIED	NO. OF HALVES
60725...	0.02	+304±41	5	+86±14	6	+76±9	12	.....	.....
61116...	0.0036	+185±19	20	+17±10	20	+53±8	20	−19±4	20

TABLE 3

GROWTH OF SECTIONS OF PEA STEMS IN AUXIN SOLUTIONS. INITIAL LENGTH 6.4 MM. EPIDERMIS OF HALF OF SECTIONS REMOVED. AVERAGES OF 6 SECTIONS. EXPERIMENT NO. 61119

SOLUTION		INCREASE IN LENGTH IN PERCENTAGE OF INITIAL LENGTH	
		AFTER 2¼ HOURS	AFTER 18 HOURS
Distilled water	With epidermis	9	17
	Without epidermis	15	22
0.03 mg. auxin per liter	With epidermis	16	28
	Without epidermis	16	22
0.3 mg. auxin per liter	With epidermis	22	39
	Without epidermis	14	22
3 mg. auxin per liter	With epidermis	23	47
	Without epidermis	12	25

In order to allow sufficient oxygen uptake, the sections were barely covered with the solution and placed in an electric shaker. The whole experiment took place in the darkroom.

The results of one experiment are given in table 3. In tap water the increase in length is more in the sections without epidermis than

in the ones with epidermis. In the auxin solutions, on the contrary, the sections with the epidermis left intact grow considerably more than the ones with the epidermis removed. The sections in water elongate because they contain a certain amount of auxin which is naturally present in the plant. The increase in length caused by this native auxin is 22 per cent in sections without epidermis, after 18 hours. In cases where the sections without epidermis have been immersed in auxin solutions the increase in length over the same period of time is also 22 per cent, except for the highest concentration where it is 25 per cent. This strongly indicates that the sections from which the epidermis has been removed do not take up hormone from the solution but grow with the hormone they contained at the time the sections were cut. Figure 2 presents the results of a similar but more elaborate experiment. The increase in length of the sections is plotted against time. The curves of the sections without epidermis in water and with epidermis in water run nearly parallel. This means that their growth rate is the same, showing that the response to auxin of the wounded sections (without epidermis) is the same as that of the sections with the epidermis left intact. Furthermore, it is clear from this figure that the growth rate of the sections with epidermis in auxin solution is considerably greater than that of the sections without epidermis in auxin solution. The growth rate of the latter is but slightly more than that of the sections in water. From the growth of the sections in water it was concluded that the response to auxin is the same in sections with and without epidermis. Hence, from the difference in growth rate between the sections with and without epidermis in auxin solutions it may be concluded that removal of the epidermis (wounding) prevents the growth substance from being taken up.

Experiments such as just described for the pea were repeated with coleoptile sections of *Avena*. The primary leaf was not removed, in order to prevent auxin from being taken up through the intact inner epidermis of the coleoptile. The results of one such experiment are presented in figure 3. It leaves no doubt that the growth rate of sections of *Avena* coleoptiles, if treated as described, is considerably less if the epidermis is removed than if it is left intact. Using sections of *Avena* coleoptiles, BONNER (**1, 2**) found that removal of the outside

epidermis increased the growth of such sections in auxin solutions. Auxin taken up through the inner epidermis (BONNER removed the

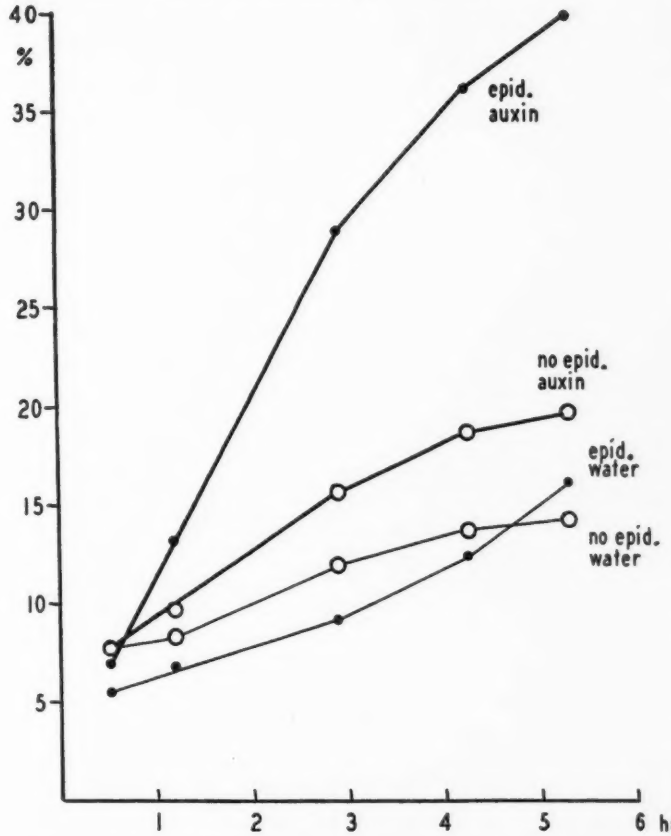


FIG. 2.—Increase in length of sections of pea stems with and without epidermis in water and in a solution containing 2.93 mg. indole-3-acetic acid per liter. Abscissa: time after sections were placed in solution. Ordinate: increase in length in percentage of original length (6.4 mm.). Experiment no. 61208.

primary leaf) must be one of the reasons why his results do not agree with ours.

A direct proof that sections from which the epidermis has been

removed cannot grow because the hormone cannot enter was made by infiltrating these sections with auxin and letting them grow again (table 4). The sections were infiltrated by sucking air out of the intercellular spaces and replacing this by an auxin solution. This was

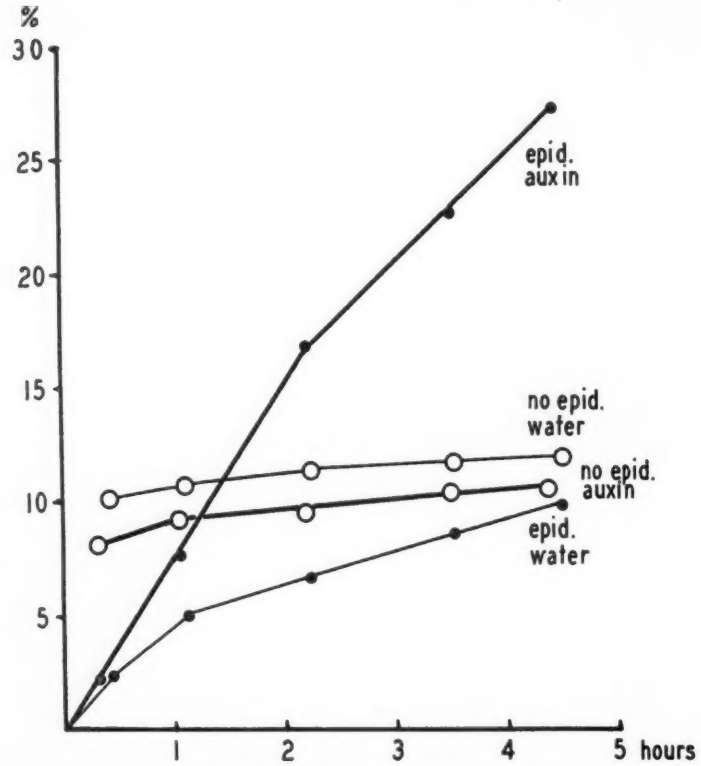


FIG. 3.—Increase in length of sections of *Avena* coleoptiles with and without epidermis in water and in solution containing 2.93 mg. indole-3-acetic acid per liter. Abscissa and ordinate same as in fig. 2. Experiment no. 61207.

done by evacuating the space above the auxin solution containing the sections and then allowing the air to enter again. Removal of the epidermis does not greatly influence growth. It is not even necessary to fill the intercellular spaces with auxin solution. Infiltration with

tap water followed by immersion of the sections in auxin solution has a similar effect. Table 5 shows that the sections of which the epidermis had been removed show the same increase in length when infiltrated with auxin and kept in an auxin solution, as they do when

TABLE 4

GROWTH OF INFILTRATED SECTIONS OF PEA STEMS. INITIAL LENGTH 6.4 MM. AVERAGES OF 12 SECTIONS. EXPERIMENT NO. 61126b

		Increase in length in percentage of initial length after				
		50 min.	2 hr.	3 hr. 45 min.	10 hr.	22 hr.
Infiltrated with water	With epidermis	1.6	3.9	9.5	18.8	21.7
	Without epidermis	3.6	7.4	10.6	17.3	19.5
Infiltrated with 3 mg. auxin per liter	With epidermis	2.5	9.8	18.0	24.8	40.0
	Without epidermis	6.0	10.6	18.5	31.0	37.3

TABLE 5

GROWTH OF INFILTRATED SECTIONS OF PEA STEMS WITH EPIDERMIS REMOVED. INITIAL LENGTH 6.4 MM. AVERAGES OF 12 SECTIONS. EXPERIMENT NO. 61202

	Increase in length in percentage of initial length after				
	15 min.	2 hr. 15 min.	4 hr. 15 min.	9 hr. 30 min.	20 hr. 30 min.
Infiltrated in water and kept in 3 mg. auxin per liter	7.0	11.2	16.1	22.8	35.6
Infiltrated with 3 mg. auxin per liter and kept in this solution	8.3	14.8	20.0	26.5	33.4

infiltrated with water followed by immersion in an auxin solution.

Next the knowledge obtained from the experiments with and without epidermis was applied to the case of the split pea stems. Here also a wound is made by splitting the stems lengthwise, and

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similarly it was to be expected that auxin could not enter through this wound into the stem. Split pea stems were infiltrated with an auxin solution or infiltrated with water and later immersed in an auxin solution. The result was that split stems either remained straight or showed a reversed pea test curvature (negatively traumatotropic). In order to prove definitely that the inside resumes growth upon infiltration, the stems were marked into segments 1 mm. long and the increase in length measured in a way similar to

TABLE 6

INCREASE IN LENGTH OF INSIDE AND OUTSIDE OF INFILTRATED SPLIT PEA STEMS DIVIDED INTO FIVE SEGMENTS, EACH HAVING INITIAL LENGTH OF 5 MM. FIGURES GIVE INCREASE IN LENGTH IN 0.1 MM. AND ARE AVERAGES OF 16 HALVES. MEASURED 18 HOURS AFTER INFILTRATION. EXPERIMENT NO. 61204

		Distance of zone below top				
		0–5 mm.	5–10 mm.	10–15 mm.	15–20 mm.	20–25 mm.
In 0.3 mg. auxin per liter	Inside	19.6±0.8 (8.7)*	18.6±0.8 (6.4)	16.3±1.0 (5.8)	11.5±1.0 (4.2)	8.4±1.1 (3.2)
	Outside	17.5±0.8 (14.8)	14.5±1.0 (14.2)	12.0±0.9 (9.4)	8.8±0.9 (6.6)	4.3±0.9 (3.0)
Infiltrated with distilled water	Inside	7.4±0.6 (2.9)	7.0±0.5 (3.5)	6.6±0.5 (2.6)	5.2±0.5 (2.9)	4.6±0.4 (2.0)
	Outside	3.4±0.5 (1.5)	3.2±0.4 (1.5)	2.4±0.4 (0.6)	1.5±0.4 (0.2)	1.1±0.3 (0.1)

* Figures in parentheses are values for increase in length of non-infiltrated split pea stems, obtained by interpolation from table 1.

that described earlier in this paper. Table 6 shows the results of this experiment. In every instance the growth of the inside was greater than that of the outside. The growth of the outside was about the same whether the split stems were infiltrated with auxin or not.

One would expect that in high auxin concentrations the auxin entering from the epidermis side would reach the wounded side and thus increase the growth on this side. This is probably the case in very high concentrations, as may be deduced from figure 6. There was also the possibility that the same agent which prevented the hormone from entering the plant through the wound also would reject the auxin from the wound inside the plant and thus cause an

unequal distribution of the hormone. In sections which were wounded on one side, however, such an unequal distribution could not be found.

Quantitative application of pea test

Technique

As suggested in a previous paper (9), the pea test can easily be adapted to quantitative auxin determinations. When Alaska peas are soaked in tap water for 6–8 hours, planted in wet sand and placed in a darkroom at 24°C. to germinate, the seedlings reach a length of about 12 to 15 cm. in the course of seven days. A rigid selection is then carried out; plants too short and too long are eliminated, and

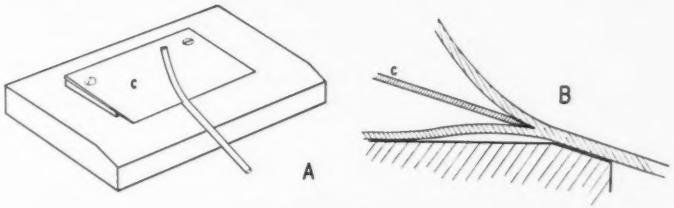

Fig. 4.—Cutter (*A*) and section through the instrument (*B*) showing position of razor blade (*c*).

only those shoots are used in which the third internode (between the highest scale or bract and the lowest leaf) is at least 40 mm. long, and the fourth internode not more than 5 mm. long. The apical 5 mm. of the stem is cut off and the stem split downward from the apical cut into two equal halves. For a uniform response it is essential to have the cut exactly median. This can be done either by hand or by means of a simple cutter (fig. 4). The split should be about 1–2 cm. longer than the growing region. Just below the end of the split the stem is cut and the split halves, which are still attached to each other at their base, are dropped in a large container filled with water to wash off the contents of the cut cells.

The solutions to be tested are made in a series of dilutions (successive concentrations differing from two to ten times), 20 cc. of which is poured in petri dishes 12 cm. in diameter. After rinsing the cut sections for about two hours, they are transferred to these dishes;

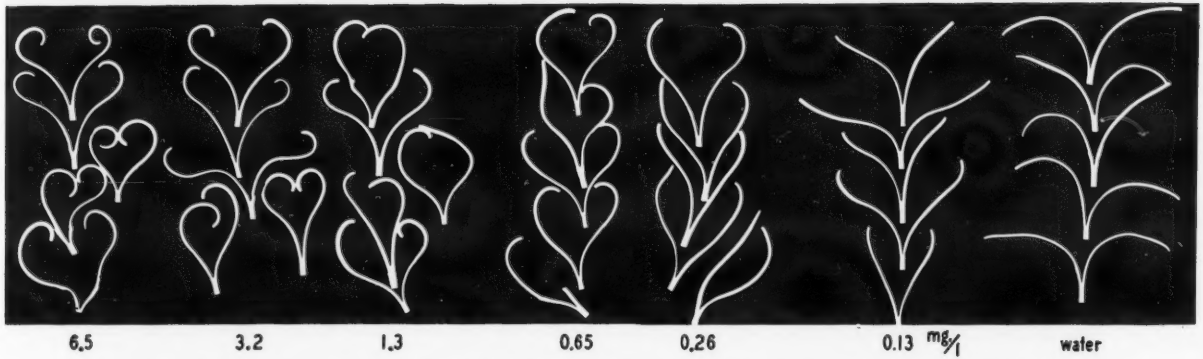

FIG. 5.—Shadow picture of slit pea stems which have been for 18 hours in different concentrations of indole-3-acetic acid, at pH 7.

five to eight, that is ten to sixteen halves, per dish. The halves are arranged so that all parts of each stem touch the solution. Under certain conditions the ends will move out of the solution, in which case it is advisable to invert them while this is happening since otherwise the parts out of the solution do not continue to curve. Within wide limits (20 minutes–6 hours) there is hardly any effect of time of washing upon the subsequent pea curvatures. With short washing periods, however, the peas do not react as uniformly as they should.

After 12 to 60 hours the curvatures are measured directly, or shadow pictures are made on bromide paper (fig. 5) and the curvatures measured later. Measurement is made with a celluloid protractor to which is attached a rotating celluloid disk with parallel lines and pointer. The protractor is first lined up with the straight region of each half (the region where the negative tissue tension curvature of the basal zones changes into the positive auxin curvature of the apical zones). The lines of the upper disk are then made parallel with the tangent of the extreme tip and the angle is read (either directly or after addition of $n \times 360$, depending on the number of loops). The curvature plotted against the log of the concentration gives in most cases a straight line. Above a certain concentration the curvatures do not increase further; at very high concentrations they decrease again (fig. 6). The relation between the concentration of a given substance and the curvature it produces can thus be expressed:

$$\alpha = K \log \frac{c}{c_0}$$

in which alpha is the observed curvature, c the concentration in moles per liter, c_0 the concentration at which the curvature is zero, and K a constant characteristic for the slope of each line. The value c_0 may be called the minimum active concentration, and denotes the point where the activity curve crosses the O line. This point is generally determined by extrapolation but may also be determined by direct measurement. The value of K denotes the rate of increase of curvature with increasing concentration. For each substance causing growth these constants are different, and thus a substance can be characterized by them.

Effect of pH on pea test curvatures

Great care should be taken to adjust all solutions to the same pH, since the auxin curvatures are very different at different pH. Only phosphate and biphthalate buffers were satisfactory; buffers requiring citric acid were unsuitable since the citric acid contained traces of active substances which caused slight bending of the split stems.

This pH effect is obvious even in the control plants in buffer solutions, which show marked differences in their behavior at different

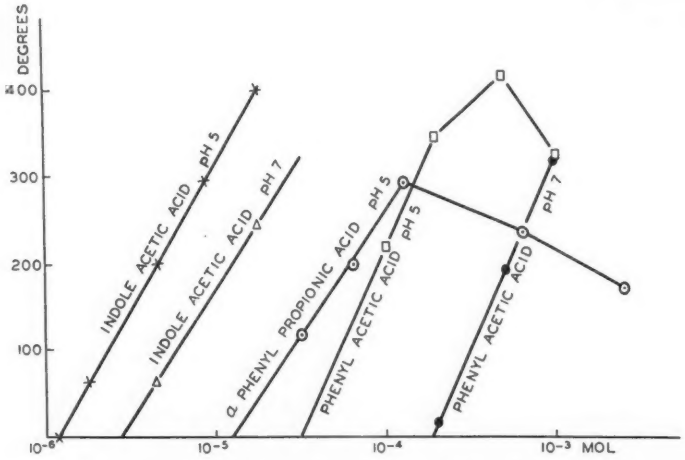

Fig. 6.—Relation between curvature (ordinate) and molar concentration (abscissa) in pea test, for various compounds at different pH.

pH, as shown by the following figures. The angle over which the split ends were bent away from their original position before cutting were 75° at pH 8; 85° at pH 7; 102° at pH 6; 125° at pH 5; and 255° at pH 4. At pH 3 the buffer is toxic, so that although the ends first rapidly curve outward they nevertheless soon lose their turgor. These are the so-called acid curvatures (**7, 1**). They are in opposite direction from the auxin curvatures, and thus the acid and auxin effects are easily distinguishable. It is the same effect as described by Bouillenne (**3**) in epidermis strips of onion leaves. Another effect of the pH on the pea test is noticed when peas are placed in the

same auxin concentration but at different pH. In concentrations giving a maximal response the effect is hardly perceptible, but in lower concentrations the curvature at a low pH is always greater than that at a higher pH. Figure 6 illustrates this for a number of compounds. The activity of each substance has been determined at pH 5 and 7. Depending upon the specific properties of each substance, the activity is decreased twofold to tenfold by decreasing the acidity from pH 5 to 7. Over the whole range of concentrations the

TABLE 7

VALUES OF C_0 AND K FOR INDOLE-3-ACETIC ACID ON DIFFERENT DAYS

	pH 5		pH 7	
	c_0	K	c_0	K
1936				
November 12	.....	.....	14.5	130
November 19	10	275	15	245
December 1	8.3	320	25	295
December 16	10	235	17	190
1937				
January 7	12	310	.....	.....
January 14	.....	.....	21	255
February 2	10	230	.....	.....
February 23	5.6	175	14	175
March 5	11	345	.....	.....
Mean	9.6±0.8	256±24	17.7±1.8	215±25

activity is generally decreased to the same extent for a particular substance. The actual relation between the physical and chemical properties of a given substance and the effect of the pH on its activity in the pea test are under further investigation.

DIURNAL AND SEASONAL CHANGES IN SENSITIVITY

There is considerable variation in the sensitivity of peas on different days. The value of K in particular changes. This is the same as in the *Avena* test, in which also diurnal and seasonal variations occur. To give an idea of the variations, table 7 lists the c_0 and K values for indole-3-acetic acid on different days. These variations are not due to a lack of uniformity of the material nor to changes of

temperature, humidity, or darkroom illumination; they depend on as yet uncontrollable external factors. These factors may influence the auxin content of the peas at the moment of splitting, the uptake of the active substance, or the sensitivity of the growing cells.

To illustrate the effect of age of the material upon the reaction, an experiment was undertaken in which each day a number of stems

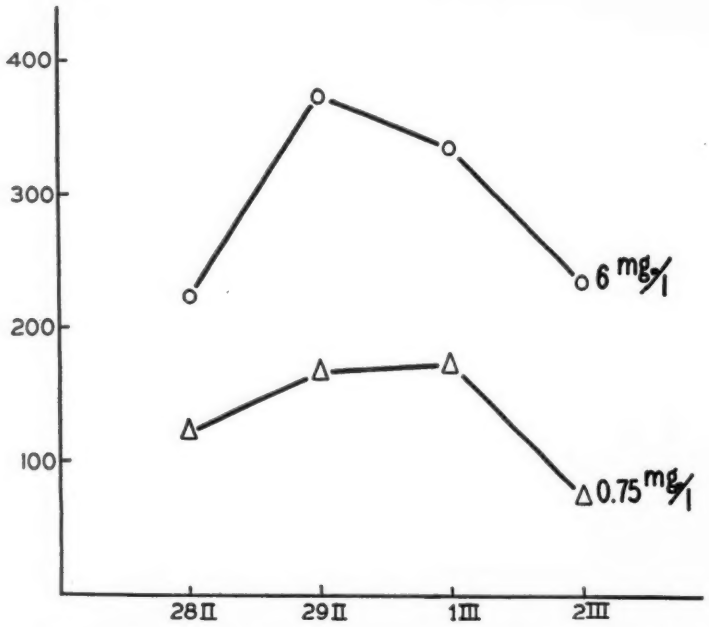

FIG. 7.—Varying sensitivity of peas from same lot on successive days. Ordinate: curvature of pea test, when split stems are treated with 6 or 0.76 mg./l. of indole-3-butyric acid.

were cut from the same set of peas. Figure 7 shows the results. The same peas, or the same internodes even, may give different reactions on successive days. In quantitative tests, therefore, a series of two or three control solutions (preferably of known concentrations of indole-3-acetic acid between 0.5 and 2 mg. per liter) should be included in order to determine the sensitivity of the plants.

Suitability of various pea races for the test

Different races of peas have been tested. Most of these were supplied by Dr. W. Brotherton, to whom our thanks are due. Of the fifty-five varieties tested, only two (Extra Early Market and Horal) were completely unsuitable, giving hardly any auxin curvatures. However, this may have been caused by the very poor growth due to infection. Eleven other varieties were not very suitable and gave moderate reactions only (some of them were dwarf varieties). The other forty-two were excellent. It does not seem necessary to list

TABLE 8

Pea test curvatures of different varieties in three concentrations of indole-3-acetic acid; all plants 8 days old when tested

Variety	Type of stem	Curvature in indole-3-acetic acid		
		3 mg./l.	1 mg./l.	0.3 mg./l.
Alaska	Long	320	196	39
Perfection	Medium long	172	30	0
Hundredfold	Medium long	124	66	30
Stratagem	Short	250	120	71
Little Marvel	Short	163	50	21
Daisy	Very short, stubby	163	92	28

them all, but Alaska, Kelvedon Wonder, Little Marvel, Charles I, and Groene Lente were among the best. In view of these results it is surprising that Jost and Reiss were not able to obtain satisfactory auxin curvatures with peas, since practically all varieties are good. In table 8 the curvatures of several pea varieties are listed. It is evident that the type of growth does not determine the suitability of the variety for the pea test.

Relation between time and curvature

It has been shown for *Avena* coleoptiles that the rate of growth (curvature) is proportional to the concentration of the auxin applied. A difference between a low and a high concentration can be found even after a time so short that the curvatures are just beginning to develop. In the pea test, however, the rate of curvature is

the same at all concentrations, as is shown in figure 8. When the auxin concentration is low the curvature proceeds for a short time only; in high concentrations the curvature continues for a much longer period.

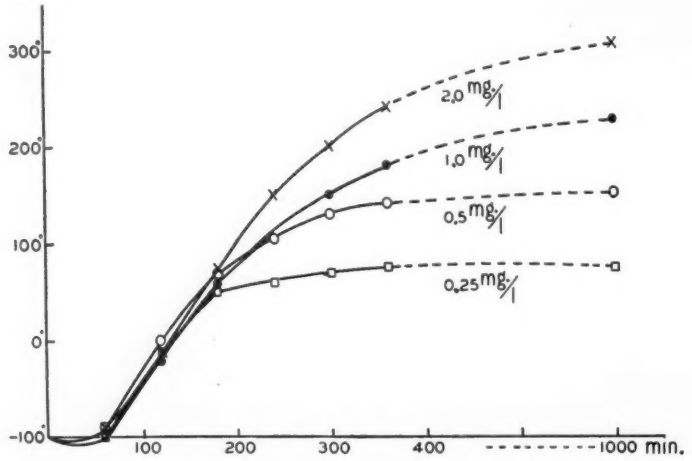

FIG. 8.—Progress of pea curvatures from moment that they are placed in indole-3-acetic acid solutions of different strength, until final curvature is reached, after 1000 minutes. Each curve represents curvature in degrees (ordinate) of fourteen halves. Initial negative curvature due to tissue tension. Rate of curvature 1–3 hours after placing stems in solution is constant, independent of final curvature.

Summary

1. If growing stems of pea seedlings are split and immersed in a solution containing auxin, the two halves will curve inward (fig. 5). This pea test curvature is proportional to the logarithm of the auxin concentration (fig. 6). The curvature is due to differential growth.

2. The epidermis side of the halved stems grows faster than the wounded inside (fig. 1). This difference in growth in turn is caused by the fact that auxin is unable to enter the stem through the wounded surface. If auxin is forced into the halved stems by infiltration with auxin solutions, the inside will grow just as much as the unwounded epidermis side (table 6).

3. The pea test can be successfully used for quantitative auxin determinations, the technique of which is described.

4. The pea test curvature is dependent upon the pH of the auxin solutions (fig. 6). At greater acidities greater curvatures are obtained. As in the *Avena* coleoptile, the sensitivity of the pea stems to auxin has a daily and seasonal variation. A wide variety of pea races was found to be suitable material for the pea test. Of the fifty-five varieties investigated only two did not give any curvature at all. The rate of curvature was shown to be independent of the auxin concentration, but the higher the concentration the longer the period of time during which the curvature proceeds.

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CHEMICAL STUDY OF THE RIPENING PROCESS OF BOSC PEARS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 481

WILLIAM E. MARTIN

(WITH FIVE FIGURES)

Introduction

In the commercial production and marketing of pears the fruit is not allowed to ripen on the tree, but is picked while it is still hard and green. Winter varieties are usually placed in storage immediately after picking and packing, and are not marketed until later in the season. Some varieties ripen slowly at storage temperatures while others remain hard and green for long periods and do not ripen until removed from storage and kept for a time at a higher temperature. The Bosc pear is one of the latter class, and tests have shown (9) that ripening temperatures of 65°–70° F. are essential for the development of best eating quality of the fruit.

The process of ripening or conditioning the fruit at these latter temperatures has become general in recent years, and much of the fruit of this variety is brought to the condition known as "firm eating ripe" before it is offered on the retail market.

The physiology of the ripening of summer pears such as the Bartlett has been investigated by several workers (1, 6, 12). There has been comparatively little work done on the chemical changes involved in the stages of ripening of winter pears taking place before the fruit is placed on the market, and no work at all, to the writer's knowledge, on the changes taking place after the fruit has attained optimum eating quality.

In the present investigation the attempt was made to determine chemically the nature of the metabolic changes taking place during the ripening of Bosc pears at 67° F. after removal from storage. The experiments were planned to determine the nature of the ripening process after the point of optimum eating quality had been reached and during the time the fruit was becoming overripe and developing

"internal breakdown." This breakdown, known also as core rot, is characterized by a brown discoloration and softening of the tissues about the core. It appears first at the stem end of the fruit along the vascular elements leading to the core and later develops in the pith parenchyma surrounding the carpels. HARLEY and FISHER (7) have demonstrated that this breakdown of the pear is associated with an accumulation of acetaldehyde in the tissues, and have suggested that after a certain concentration of acetaldehyde is reached browning and breakdown take place.

Bosc pears taste very much sweeter at the time of optimum eating quality than they do when either green or overripe. It was planned, therefore, to determine accurately whether there was any appreciable increase in sugars during ripening or whether the observed increase in sweetness was merely caused by a concentration of solutes as the fruit lost water by transpiration.

The experiment was carried out with three different lots of fruit, one picked at the time of commercial harvest, another ten days earlier, and the third ten days after the commercial crop. A number of representative samples which had received the same treatment as the commercial crop were placed in a ripening room at 67° F., and samples were withdrawn for analysis on each of twenty successive days. At the time the fruit was placed in the ripening room the pears of all three pickings were still hard. The green ground color was beginning to fade and patches of yellow were appearing in the fruit of the late picking.

Material and methods

The fruit used in this investigation was obtained through the courtesy of F. C. REIMER of the Southern Oregon Experiment Station, Talent, Oregon, and was picked from a single Bosc tree.

Three pickings of four boxes each were made on September 3, 13, and 23, 1935. The picking on September 13 corresponded with the commercial harvest of Bosc pears in the region; the other pickings were respectively earlier and later. The maturity of the fruits was measured by the Oregon pressure tester (13) and the results of these measurements are listed in table 1. The pressure test is based on the fact that during the growth and ripening of the pear there is a

gradual and consistent lowering of the physical resistance to pressure or wounding of the epidermal and cortical regions of the fruit.

Immediately after picking, the fruit was wrapped and packed in boxes and placed in storage at 30°–31° F., and on November 16 it was removed from storage and shipped to Chicago under ventilation, arriving November 25. After arrival, the fruit was removed immediately from the shipping boxes and graded on the basis of size. All fruits injured by stem punctures or damaged by insects were discarded.

The fruit of each picking was then divided into twenty representative lots containing an equal number of large, medium, and small pears. In series A there were twenty pears in each of the twenty

TABLE 1

MATURITY OF PEARS AT TIME OF PICKING

SERIES	PICKED	PRESSURE IN POUNDS
A	September 3	24.8
B	September 13	21.3
C	September 23	17.9

sample lots put up for ripening. The fruits of the second and third pickings (series B and C) were somewhat larger and only eighteen representative pears were taken for each sample lot. Thus the fruit of each picking was divided into twenty representative sample lots of eighteen or twenty pears each. All of the sixty samples thus prepared were weighed and placed in a ripening room in which the temperature was maintained at 67° F.

Three sample lots, one of each series, were removed from the ripening room on each of 20 successive days. Thus three series of samples were obtained and each series represented all stages of ripeness, ranging from the green condition to fruit of good eating quality and continuing on to overripeness and "internal breakdown" owing to natural causes. It was planned to obtain samples which would yield data, not only on the sequence of chemical events involved in ripening, but also on the effects of time of picking upon the ripening process.

It was found necessary to modify the experiment somewhat, since

it was desirable to discard all sample lots which had decayed during the ripening period. Daily samples were taken for 17–20 days, depending upon the number of undecayed sample lots available in each case.

Methods of analysis

At the time of removal from the ripening room the fruit was again weighed carefully and the loss of weight during the period of ripening computed for each of the sample lots examined.

Following weighing, samples of each lot were taken for analysis. This was accomplished by cutting two opposite longitudinal sectors from each pear, grinding the sectors through a Russwin food chopper, and then weighing duplicate 50 gm. samples from the ground pulp. These samples were preserved by boiling with sufficient 95 per cent ethyl alcohol to obtain a final concentration of 80 to 85 per cent. The preserved samples were then set aside for chemical analysis.

At the time samples were taken for analysis an attempt was made to gauge roughly the eating quality of the fruit. While such tests were obviously inaccurate, approximate data were obtained as to the time in the sequence of sampling at which the fruit was of good eating quality.

Extraction.—This was carried out with the Soxhlet apparatus. The preserved samples were transferred to a weighed filter paper folded into a paper extraction cup and the filtrate collected in a volumetric flask. The extraction cup and contents were then placed in the Soxhlet apparatus and extracted with 95 per cent ethyl alcohol for at least 24 hours and until the liquid in the upper receiver showed a negative alpha-naphthol carbohydrate test. The extract was then added to the original alcoholic filtrate and the sample made up to 500 ml. with 95 per cent ethyl alcohol. The solution thus prepared contained the alcohol soluble solids.

Alcohol soluble solids.—These were determined gravimetrically by pipetting an aliquot of the alcohol extract into a tared 100 ml. beaker, evaporating the alcohol on the steam bath, and finally drying in a vacuum oven at 75° C. After 65 hours' drying approximately constant weight had been reached and the residue was then weighed.

ALCOHOL INSOLUBLE RESIDUE.—The residue was determined similarly by drying the tared filter paper and contents after removal from the Soxhlet apparatus. The initial weight of the filter paper and the final weight of the filter paper and residue were both obtained by drying to constant weight at 75° C. in the vacuum oven. All weighings were made in large weighing bottles to prevent absorption of moisture by the dry filter papers and residues.

TOTAL SOLIDS.—These were calculated as the sum of the alcohol soluble solids and the alcohol insoluble residue.

SUGAR DETERMINATIONS.—Determinations were made in such a way as to yield the relative amounts of sucrose, dextrose, and levulose present in the pear. Aliquot fractions of the alcohol extracts were pipetted into 250 ml. volumetric flasks and the alcohol evaporated on the steam bath. This was readily accomplished by inserting glass tubes into the necks of the flasks and blowing a stream of air on the liquid while heat was applied from below. The alcohol evaporated readily and was replaced by two additions of distilled water, 25 ml. each, after which the volume was finally reduced to about 10 ml.

The aqueous extract thus obtained was cleared by the addition of 5 ml. of a saturated solution of neutral lead acetate, deleaded with a slight excess of potassium oxalate, made up to volume, and filtered at once into a dry flask. The clear solution thus obtained was then ready for the determination of reducing sugars before inversion.

A separate solution was prepared for the determination of reducing sugars after inversion. The procedure was the same as the preceding, except that the aqueous extract before clarification was made up to about 50 ml., acidified with five drops of 10 per cent acetic acid and inverted by five drops of a 1 per cent solution of invertase scales. The reaction mixture was allowed to stand overnight at room temperature and then cleared, deleaded, and filtered as described.

REDUCING SUGARS BEFORE AND AFTER INVERSION.—These were determined by a modification of the Official Methods (**2**). Reduction was carried out according to the conditions of Munson and Walker, and the Cu_2O collected on asbestos pads in Gooch crucibles. The pad and Cu_2O were then suspended in 25 ml. of hot water and

the Cu_2O dissolved by heating after adding 5 ml. of 50 per cent sulphuric acid and a slight excess of a saturated solution of potassium permanganate. The excess permanganate was then decomposed by adding a saturated solution of potassium oxalate dropwise until the color of the permanganate disappeared and the pale blue of the cupric sulphate remained. The excess of the oxalate solution was limited to one drop. After cooling, 10 ml. of a 30 per cent solution of potassium iodide was added and the liberated iodine titrated with 0.05 N sodium thiosulphate. One ml. of 0.05 N sodium thiosulphate is equivalent to 3.597 mg. of Cu_2O. The sugar equivalent to the Cu_2O was obtained from the Munson and Walker tables and was expressed both as invert sugar and as dextrose.

SUCROSE.—This was calculated from the difference in reducing sugars before and after inversion, and is expressed as sucrose.

DEXTROSE AND LEVULOSE.—These were determined by the method of LOTHROP and HOLMES (11), and the determinations were carried out on samples drawn from the same solutions that were prepared for the determination of reducing sugars before inversion.

TOTAL SUGARS.—These were calculated as the numerical sum of the determined amounts of sucrose, dextrose, and levulose.

NON-SUGAR SOLUBLE SOLIDS.—These were calculated as the difference between the alcohol soluble solids and the total sugars.

SORBITOL.—This was determined by a modification of WERDER'S method (18). This type of method depends on the formation of dibenzal-sorbitol from sorbitol and benzaldehyde in the presence of strong sulphuric acid as a condensing agent. In order to obtain quantitative yields of dibenzal-sorbitol the proportions of sorbitol, benzaldehyde, and sulphuric acid must be carefully controlled. Similarly the time and temperature of condensation and the method of treatment of the precipitated dibenzal-sorbitol must be judiciously carried out if approximately theoretical yields are to be obtained.

Several quantitative methods were tried out using pure sorbitol (Pfanstiehl). The method of BLEYER, DIEMAIR, and LIX (3) was found most satisfactory. Approximately quantitative yields of dibenzal-sorbitol were obtained if the precaution was taken of nentralizing the sulphuric acid with a slight excess of sodium carbonate, bringing to a boil, and finally cooling before filtering off the dibenzal,

drying and weighing. It was found that the dibenzal-sorbitol so treated could be dried at 100° C. without decomposition and that after 10–12 hours a constant weight had been attained.

The general method and proportions of the reagents used in the determination of sorbitol were essentially those listed by BLEYER, DIEMAIR, and LIX in their modification of WERDER'S original procedure, but certain further modifications were made and the procedure used in this investigation is briefly described.

About 75 ml. of the alcohol extract containing the soluble solids was pipetted into a flask and 1 gm. of Mallinkrodt activated charcoal was added. The flask was tightly corked, well shaken, and the solution filtered at once through a fluted filter paper (Whatman no. 42) into a dry flask. The filtration was rapid and within 10–15 minutes a 50 ml. aliquot of the clear filtrate was pipetted into a 100 ml. beaker and evaporated on the steam bath to a very thick syrup, which solidified on cooling.

One ml. of 50 per cent sulphuric acid (1:1) and 0.5 ml. of benzaldehyde were added and the mixture stirred well for about 5 minutes and then placed in the refrigerator (1°–3° C.) for 19–20 hours. During this time a thick yellowish white paste was formed. Fifty ml. of cold distilled water was then added and the paste broken up with a stirring rod. After remaining in the refrigerator for two hours longer a slight excess of anhydrous sodium carbonate was added (1.5 gm.). Finally one hour later the material was removed from the refrigerator, boiled for one to two minutes, and cooled in running water. The white amorphous precipitate of dibenzal-sorbitol was then collected in a tared gooch crucible, washed thoroughly with distilled water, and weighed after drying 10–12 hours at 100° C. One hundred mg. of sorbitol is equivalent theoretically to 196.8 mg. of dibenzal-sorbitol.

This method was found to give approximately quantitative yields in the range between 60 and 250 mg. of sorbitol per determination. The results of chemical tests following this procedure are shown in table 2.

The necessity of removal of sugars before determining sorbitol has been stressed by several workers (**3, 15**), who have reported low yields of dibenzal-sorbitol if sugars were not removed. In this in-

TABLE 2

QUANTITATIVE DETERMINATION OF SORBITOL

SORBITOL TAKEN	WEIGHT OF DIBENZAL-SORBITOL		PERCENTAGE YIELD
	OBTAINED	THEORETICAL	
75	148.8	147.6	100.8
75	149.9	147.6	100.6
100	198.0	196.8	100.6
100	200.0	196.8	101.6
125	240.2	246.0	97.6
125	240.0	246.0	97.6
150	277.4	295.2	83.8
150	267.4	295.2	90.6
175	346.7	344.4	100.7
175	344.7	344.4	100.1
200	395.2	393.6	100.4
200	398.9	393.6	101.3
250	492.1	492.0	100.0
250	492.1	492.0	99.8

TABLE 3

EFFECTS OF SUGARS ON DETERMINATION OF SORBITOL

SORBITOL TAKEN (MG.)	SORBITOL FOUND			
	SORBITOL ALONE		SORBITOL AND SUGAR*	
	WEIGHT (MG.)	YIELD (%)	WEIGHT (MG.)	YIELD (%)
75	78.7	104.4	77.6	103.2
100	105.8	105.8	105.8	105.8
125	134.8	107.8	131.0	105.0
150	144.4	96.3	154.4	103.0
175	168.0	96.0	171.3	97.8
200	184.6	92.5	191.1	96.4

* The same amount of dextrose, sucrose, and levulose was added to each sample as was present in a 50 ml. aliquot of the extract of sample B-6.

vestigation, however, it was found that if condensation were allowed to proceed for 19–20 hours in the refrigerator at 1°–3° C. approximately quantitative yields of the dibenzal derivative were obtained from the analysis of solutions of pure sorbitol to which definite amounts of sugar had been added. The results of such tests are shown in table 3. The amounts of dextrose, levulose, and sucrose added to each sample were approximately the same as had been determined to be present in 50 ml. aliquots of pear extracts.

It may be seen from table 3 that the method used in this investigation is approximately quantitative and that errors did not exceed 8 per cent of correct values. Tests with much larger amounts of dextrose added alone to pure sorbitol showed less effect on the determination of sorbitol than shown in table 3.

Presentation of data

Loss of weight during ripening

The relative weight losses of pears of the three different pickings are shown in table 4 and in figure 1. These losses are expressed as percentages of the original weight of the fruit at the time it was placed in the ripening room, and represent the sum of the water loss, the carbon dioxide loss, and the loss due to other volatile products evolved by the fruit during metabolism.

It may be noted that the fruit of series B, picked September 13, lost weight somewhat more rapidly than did the fruit of series A and C which were picked on September 3 and 23 respectively. The magnitude of the weight loss was about 0.5 per cent per day in the fruit of series A and C, which corresponds approximately to the weight losses reported by Hartman (**8**) in his investigation of Bartlett pears held at 66° F. In over 400 hours there was no apparent reduction of the rate of weight loss, except perhaps in series B where a larger percentage of the original weight had been lost.

Composition of pears at various stages of ripening

The data presented in table 5 show the changes in the concentration of sugars and other constituents of the fruit of the three pickings as measured by the composition of individual sample lots

taken at daily intervals during the ripening period. These data are expressed as percentages of the fresh weight of the fruit at the time of withdrawal from the ripening room and thus give a measure of the composition of the fruit in so far as determined in this investigation.

TABLE 4

EFFECT OF TIME OF PICKING ON LOSS OF WEIGHT DURING RIPENING

Series A September 3, 1935			Series B September 13, 1935			Series C September 23, 1935		
Hours ripened	Loss of weight (%)	Part of original weight*	Hours ripened	Loss of weight (%)	Part of original weight*	Hours ripened	Loss of weight (%)	Part of original weight*
13	0.36	0.9964	22	0.76	0.9924	25	0.65	0.9935
37	0.96	0.9904	46	1.67	0.9833	48	1.29	0.9871
61	1.39	0.9861	62	2.05	0.9795	70	1.36	0.9864
81	2.09	0.9791	94	2.95	0.9705	95	1.75	0.9825
118	2.86	0.9714	121	3.54	0.9646	122	2.78	0.9722
141	3.52	0.9648	143	4.32	0.9568	145	2.90	0.9710
166	3.96	0.9604	166	5.08	0.9492	169	3.24	0.9676
192	4.35	0.9565	192	5.91	0.9409	191	4.24	0.9576
214	5.05	0.9495	213	7.11	0.9289	213	4.82	0.9518
234	4.94	0.9506	237	7.84	0.9216	235	4.73	0.9627
260	5.72	0.9428	263	8.33	0.9167	265	5.39	0.9461
285	6.38	0.9362	286	8.18	0.9182	287	5.55	0.9445
308	7.04	0.9296	309	8.59	0.9141	309	6.85	0.9315
325	7.15	0.9285	331	9.48	0.9052	333	7.44	0.9256
360	7.83	0.9217	360	10.09	0.8991	361	7.84	0.9216
384	8.46	0.9154	384	11.81	0.8819	408	.....	.....
405	.....	.....	404	11.02	0.8894	457	9.89	0.9011
432	10.10	0.8990	.....	.....	.....	.....	.....	.....
480	10.83	0.8917	.....	.....	.....	.....	.....	.....

* Part or proportion of original weight remaining.

The data obtained from the three different sets of fruit, picked ten days apart, show very similar composition and exhibit the same trends during the ripening process. Owing to this similarity only the data of series B are presented (fig. 2).

It may be seen from figure 2 that there was a definite increase in the total sugar content of the fruit as it ripened and that this increase continued throughout the whole period observed. The concentration of sucrose increased for the first six days of ripening and then decreased rapidly and continually for the remainder of the

period. The concentration of dextrose remained approximately constant until the decrease in sucrose was observed, and then increased as the amount of sucrose became less. Levulose increased in concentration steadily during the whole of the ripening process.

Fruit of good eating quality was obtained between the sixth and tenth day. Thereafter all fruit was inferior and internal breakdown became increasingly widespread from the eleventh day to the end of the period.

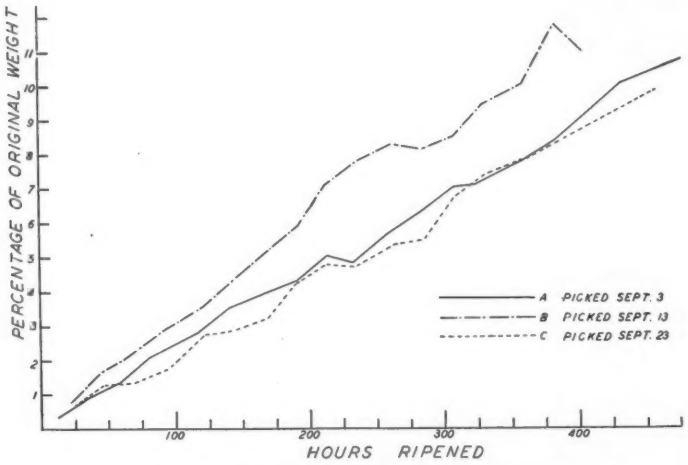


FIG. 1.—Weight loss during ripening of Bosc pears.

There were considerable fluctuations in the observed values of total solids and these fluctuations caused a marked scatter in the points upon the total solids curve as shown in figure 2.

The interpretation of data expressed in terms of fresh weight at the time of sampling may lead to erroneous conclusions, as will be shown later. The data shown in figure 2 and table 5 are presented, however, since they represent a measure of the actual composition, and since most of the American work on the physiology of fruit ripening has been expressed on this basis.

TABLE 5

COMPOSITION OF BOSC PEARS EXPRESSED AS PERCENTAGE FRESH WEIGHT AT TIME OF SAMPLING

DAYS RIPENED	DEXTROSE	LEVULOSE	SUCROSE	TOTAL SUGARS	NON-SUGAR SOLUBLE SOLIDS	SORBITOL	SOLUBLE SOLIDS	ALCOHOL INS. RES.	TOTAL SOLIDS
	Series A, picked September 3, 1935								
1	1.95	5.47	1.93	9.35	4.54	3.73	13.89	4.30	18.19
2	1.64	5.76	2.38	9.78	4.31	3.54	14.09	4.04	18.13
3	1.95	5.47	2.08	9.50	3.99	3.18	13.49	3.94	17.43
4	1.99	5.74	2.67	10.40	4.26	3.08	14.66	4.13	18.79
5	1.76	6.06	2.62	10.44	3.46	2.87	13.90	3.81	17.71
6	1.70	6.05	2.60	10.35	3.25	2.61	13.60	3.62	17.22
7	2.09	6.16	2.02	10.27	3.54	2.56	13.81	3.67	17.48
8	2.20	6.11	1.99	10.30	3.36	2.39	13.66	3.87	17.53
9	2.29	6.61	2.29	11.19	2.96	2.33	14.15	3.90	18.05
10	2.30	6.69	2.19	11.18	2.81	2.23	13.99	3.68	17.67
11	.....	.....	1.62	.....	.....	2.17	13.53	3.41	16.94
12	2.40	6.71	1.48	10.59	2.59	2.09	13.18	3.77	16.95
13	2.57	6.88	1.08	10.53	2.78	.....	13.31	3.56	16.87
14	2.63	7.36	1.00	10.99	3.06	1.98	14.05	3.52	17.57
15	2.72	7.31	.86	10.89	2.90	.....	13.79	3.79	17.58
16	2.79	7.16	.81	10.76	2.93	1.89	13.69	3.69	17.38
17	2.86	7.54	.81	11.21	2.97	.....	14.18	3.74	17.92
18	2.97	7.76	.71	11.44	3.00	1.98	14.44	3.96	18.40
20	2.93	7.56	.66	11.15	2.87	1.93	14.02	3.88	17.90
	Series B, picked September 13, 1935								
1	1.68	5.06	2.94	9.68	4.23	3.31	13.91	3.31	17.56
2	1.67	5.25	3.12	10.04	4.25	3.24	14.29	3.79	18.08
3	1.69	5.08	3.51	10.28	3.80	2.99	14.08	3.84	17.92
4	1.68	5.56	3.70	10.94	3.82	2.88	14.76	3.71	18.47
5	1.66	5.59	3.79	11.04	3.77	2.71	14.81	3.60	18.41
6	1.71	5.76	3.90	11.37	3.29	2.59	14.66	3.89	18.55
7	1.85	5.90	3.77	11.52	3.31	2.32	14.83	3.77	18.60
8	1.88	6.22	3.37	11.47	3.20	2.11	14.67	3.73	18.40
9	1.92	6.38	3.68	11.98	3.14	2.21	15.12	3.63	18.75
10	2.11	6.45	2.94	11.50	3.17	2.15	14.67	3.88	18.55
11	2.24	6.88	3.18	12.30	3.23	2.17	15.53	3.67	19.20
12	2.57	6.97	2.54	12.08	3.10	2.18	15.18	3.90	19.08
13	2.60	6.98	2.22	11.80	2.99	1.94	14.79	3.81	18.60
14	2.75	7.04	2.00	11.79	2.88	1.95	14.67	3.86	18.53
15	2.96	7.12	1.90	11.98	3.10	1.97	15.08	3.97	19.05
16	3.04	7.65	1.71	12.40	2.99	1.98	15.39	3.91	19.30
17	3.28	7.72	1.68	12.68	2.90	.....	15.58	3.68	19.26

TABLE 5—*Continued*

Days ripened	Dextrose	Levulose	Sucrose	Total sugars	Non-sugar soluble solids	Sorbitol	Soluble solids	Alcohol ins. res.	Total solids
	Series C, picked September 23, 1935								
3	1.60	5.26	4.15	11.01	3.98	2.90	14.99	3.41	18.40
4	1.48	5.49	4.47	11.44	4.03	3.07	15.47	3.36	18.83
5	1.54	5.46	4.31	11.31	3.53	2.58	14.84	3.37	18.21
6	1.55	5.78	4.37	11.70	3.52	2.41	15.22	3.35	18.57
7	1.55	5.89	4.74	12.18	3.51	2.46	15.69	3.42	19.11
8	1.76	6.17	4.13	12.06	3.36	2.12	15.42	3.31	18.73
9	1.89	6.41	4.10	12.40	3.42	2.19	15.82	3.28	19.10
10	2.03	6.58	3.72	12.33	3.37	.....	15.70	3.45	19.15
11	2.33	6.63	3.21	12.17	3.45	2.08	15.62	3.22	18.84
12	2.49	7.03	2.70	12.22	3.12	2.05	15.34	3.24	18.58
13	2.80	6.80	2.94	12.54	2.80	1.90	15.34	3.35	18.69
14	2.84	7.48	2.62	12.94	2.98	1.91	15.92	3.36	19.28
15	2.91	7.45	2.20	12.56	2.93	1.93	15.49	3.31	18.80
17	3.23	7.94	1.39	12.56	2.60	1.94	15.16	3.40	18.56
19	3.43	8.00	1.23	12.66	2.80	1.85	15.46	3.57	19.03

Chemical changes taking place during ripening

The results presented in the preceding section were listed as percentages of the fresh weight at the time the samples were removed from the ripening room. In a quantitative study of chemical changes in apples, Haynes and Archbold (10) have shown that results expressed in terms of fresh weight at the time of sampling do not necessarily show the actual changes in the various constituents of the fruit, and that, if a correct picture of the metabolic process is to be obtained, results should be expressed in terms of original fresh weight.

It was shown earlier in this paper that marked losses of weight took place as the fruit ripened. These losses amounted to about 0.5 per cent of the original fresh weight per day and were probably largely water. It might be expected that the concentration of solutes in the cell sap would increase as evaporation of water took place. By expressing results as percentages of fresh weight an increase in sugars might be observed, although no actual synthesis had taken place and the sugar already present merely had come to represent an increasing proportion of the fresh weight of the fruit.

In series B, in which the amounts of total sugars definitely increased when expressed as percentages of fresh weight at the time

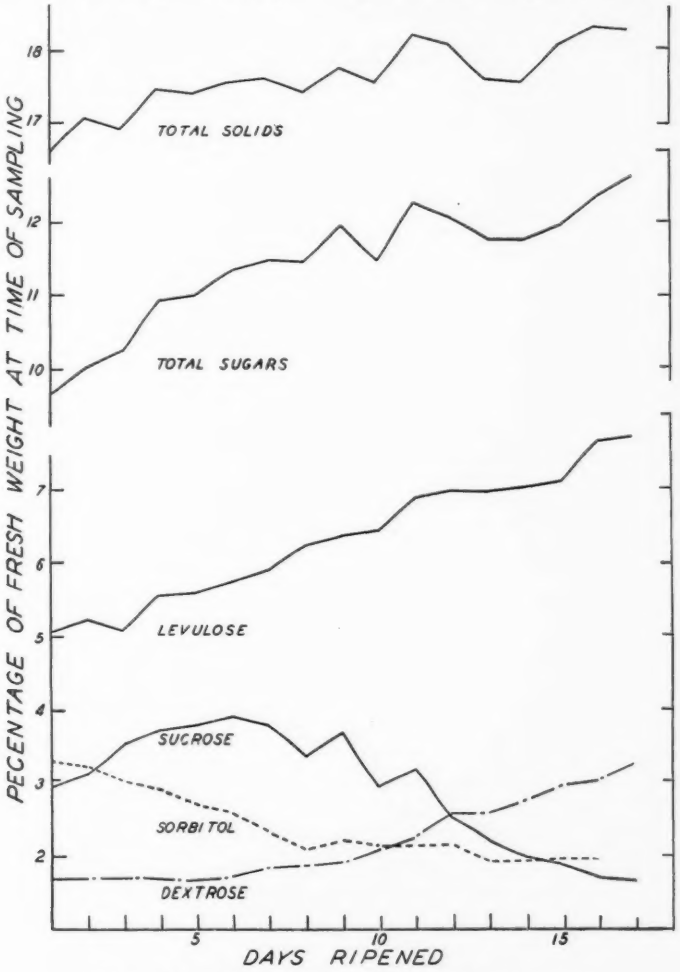


FIG. 2.—Changes in composition of Bosc pears during ripening.

of sampling, an apparent increase in total solids was also observed, as may be seen from figure 2. It seems unlikely, however, that the

amount of solid material in a pear should increase appreciably during ripening, especially in view of the fact that there should be decreases in total solids owing to losses of carbon dioxide and other volatile products of the metabolism of the fruit.

This apparent discrepancy may be explained if we recall that 11 per cent of the weight of the fruit was lost, leaving only 89 per cent of the original weight of the sample after seventeen days of ripening. Thus a 50 gm. sample taken after seventeen days represented a considerably larger proportion of the fruit than did a 50 gm. sample at the beginning of the tests. If a 100 gm. fruit were analyzed, the 50 gm. sample taken at the start would represent one half of the fruit, but after seventeen days would represent $\frac{50}{89}$ of the fruit. If results were expressed only in terms of fresh weight a comparison might be made between 100 gm. of original fruit and tissue equivalent to 112 gm. when analysis was made seventeen days later. To correct for this error in the effective size of sample, the results may be multiplied by 0.89, which is the value of the proportion of original fresh weight remaining after seventeen days of ripening. After carrying out this calculation it is found that total solids at the start of ripening represented 17.43 per cent of the original fresh weight, while after seventeen days the total solids were only 17.14 per cent of the original fresh weight of the fruit.

Thus there was actually a slight decrease in the total solid material during ripening, whereas the calculation of results in terms of fresh weight at time of sampling showed an increase in total solids.

In order to ascertain the actual changes in the various constituents of the fruit as ripening took place, the data shown in table 5 were recalculated as percentages of the original fresh weight. This was accomplished by multiplying the values listed in the table by the corresponding values of "proportion of original weight remaining" which are listed in table 4. By these calculations all data were reduced to a comparable basis, percentages of original fresh weight, and have been so listed in table 6. As before, only the data of series B are presented (fig. 3), since the changes shown by the fruit of this series appear to be representative of all three sets studied.

CARBOHYDRATE CHANGES IN RIPENING.—The data plotted in figure 3 show a definite increase in levulose throughout the whole

TABLE 6

CHANGES IN CONSTITUENTS OF BOSC PEARS EXPRESSED AS PERCENTAGE ORIGINAL FRESH WEIGHT

DAYS RIPENED	DEXTROSE	LEVULOSE	SUCROSE	TOTAL SUGARS	NON-SUGAR SOLUBLE SOLIDS	SORBITOL	TOTAL SOLUBLE SOLIDS	ALCOHOL INS. RES.	TOTAL SOLIDS
	Series A, picked September 3, 1935								
1	1.94	5.45	1.93	9.32	4.52	3.72	13.84	4.28	18.12
2	1.62	5.70	2.36	9.68	4.27	3.51	13.95	4.00	17.95
3	1.92	5.39	2.06	9.37	3.93	3.14	13.30	3.88	17.18
4	1.95	5.62	2.61	10.18	4.17	3.02	14.35	4.05	18.40
5	1.71	5.88	2.55	10.14	3.36	2.79	13.50	3.70	17.20
6	1.64	5.84	2.51	9.99	3.13	2.52	13.12	3.49	16.61
7	2.01	5.92	1.94	9.87	3.40	2.46	13.27	3.52	16.79
8	2.10	5.85	1.90	9.85	3.22	2.29	13.07	3.70	16.77
9	2.17	6.28	2.17	10.62	2.81	2.21	13.43	3.70	17.13
10	2.19	6.36	2.08	10.63	2.67	2.12	13.30	3.50	16.80
11	.....	.....	1.53	.....	.....	2.05	12.76	3.21	15.97
12	2.25	6.28	1.39	9.92	2.42	1.96	12.34	3.53	15.87
13	2.40	6.40	1.00	9.80	2.58	.....	12.38	3.30	15.68
14	2.44	6.83	.93	10.20	2.84	1.84	13.04	3.27	16.31
15	2.51	6.74	.79	10.04	2.67	.....	12.71	3.49	16.20
16	2.55	6.55	.75	9.85	2.68	1.73	12.53	3.38	15.91
18	2.67	6.98	.64	10.29	2.70	1.78	12.99	3.55	16.54
20	2.61	6.74	.59	9.94	2.56	1.72	12.50	3.46	15.96
	Series B, picked September 13, 1935								
1	1.67	5.02	2.92	9.61	4.20	3.29	13.80	3.62	17.42
2	1.64	5.16	3.07	9.87	4.18	3.19	14.05	3.73	17.78
3	1.65	4.98	3.44	10.07	3.72	2.93	13.79	3.76	17.55
4	1.63	5.40	3.59	10.62	3.71	2.80	14.33	3.60	17.93
5	1.60	5.39	3.66	10.65	3.64	2.61	14.29	3.47	17.76
6	1.64	5.51	3.73	10.88	3.15	2.48	14.03	3.72	17.75
7	1.76	5.60	3.58	10.94	3.14	2.20	14.08	3.58	17.66
8	1.77	5.85	3.17	10.79	3.01	1.98	13.80	3.51	17.31
9	1.78	5.93	3.42	11.13	2.92	2.05	14.05	3.37	17.42
10	1.94	5.94	2.72	10.60	2.92	1.98	13.52	3.58	17.10
11	2.05	6.31	2.92	11.28	2.96	1.99	14.24	3.36	17.60
12	2.36	6.40	2.33	11.09	2.85	2.00	13.94	3.58	17.52
13	2.38	6.38	2.03	10.79	2.73	1.77	13.52	3.48	17.00
14	2.49	6.37	1.81	10.67	2.61	1.77	13.28	3.49	16.77
15	2.66	6.40	1.71	10.77	2.79	1.77	13.56	3.57	17.13
16	2.68	6.74	1.51	10.93	2.64	1.75	13.57	3.45	17.02
17	2.92	6.87	1.49	11.28	2.58	.....	13.86	3.27	17.13

TABLE 6—*Continued*

Days ripened	Dextrose	Levulose	Sucrose	Total sugars	Non-sugar soluble solids	Sorbitol	Total soluble solids	Alcohol ins. res.	Total solids
	Series C, picked September 23, 1935								
3.....	1.58	5.19	4.09	10.86	3.93	2.86	14.79	3.36	18.15
4.....	1.46	5.39	4.39	11.24	3.96	3.02	15.20	3.30	18.50
5.....	1.50	5.31	4.19	11.00	3.43	2.51	14.43	3.28	17.70
6.....	1.51	5.61	4.24	11.36	3.42	2.34	14.78	3.25	18.03
7.....	1.50	5.70	4.59	11.79	3.40	2.38	15.19	3.30	18.49
8.....	1.69	5.91	3.95	11.55	3.22	2.03	14.77	3.17	17.94
9.....	1.80	6.10	3.90	11.80	3.26	2.08	15.06	3.12	18.18
10.....	1.93	6.27	3.54	11.74	3.21	.....	14.95	3.29	18.24
11.....	2.20	6.27	3.04	11.51	3.26	1.97	14.77	3.05	17.82
12.....	2.35	6.64	2.55	11.54	2.95	1.94	14.49	3.06	17.55
13.....	2.61	6.33	2.74	11.68	2.61	1.77	14.29	3.12	17.41
14.....	2.63	6.92	2.43	11.98	2.76	1.77	14.74	3.11	17.85
15.....	2.68	6.87	2.03	11.58	2.70	1.78	14.28	3.05	17.33
19.....	3.09	7.21	1.11	11.31	2.52	1.67	13.93	3.22	17.15

of the ripening process. Sucrose increased during the first six days and then decreased steadily for the remainder of the period. While sucrose was increasing, dextrose remained constant; but as sucrose was hydrolyzed, dextrose appeared to be formed. As might be expected, the increases in dextrose and levulose accompanying the decrease in sucrose were approximately equal.

The points on the graphs show considerable scatter and it is difficult to judge the exact slope of the curves in some cases. The general trends may be observed, however, and a fair approximation made of the magnitude of the changes in the several sugars present.

Total sugars were found to increase definitely for the first six days; beyond that point little change could be observed with certainty owing to the scatter of the points on the curve. Total solids decreased during the ripening process. As has already been pointed out, this finding is more understandable than that observed when results were calculated as percentages of the fresh weight at the time of sampling.

The data for series A and C, the early and late picked fruit respectively, show essentially the same results as have been described,

except that the increases in total sugars were less marked and the decreases in total solids somewhat greater.

The early picked fruit contained less sucrose at all stages of ripe-

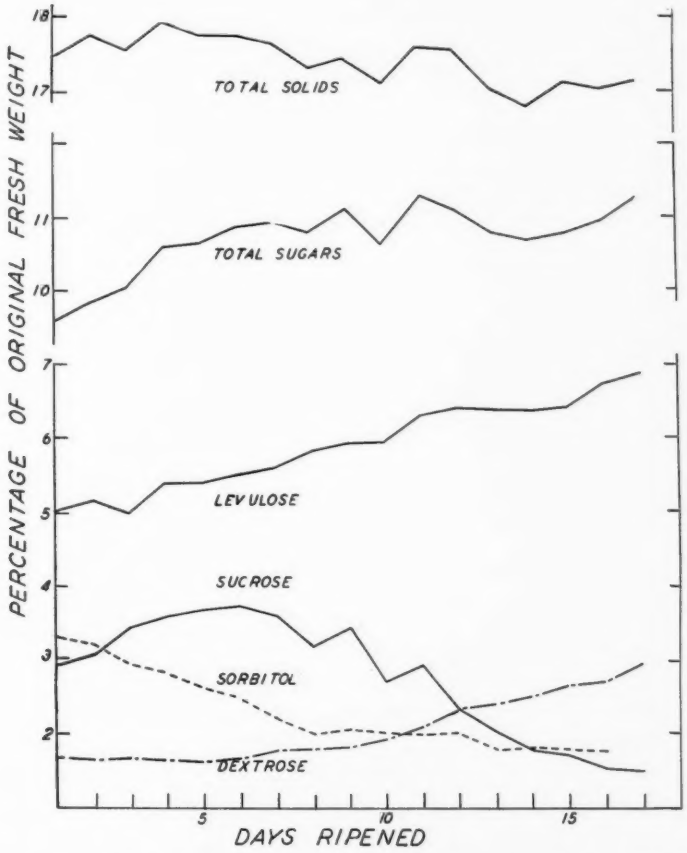


FIG. 3.—Changes which take place in the constituents of Bosc pears during ripening.

ness than did the fruit of the commercial picking. On the other hand, the late picked fruit showed more sucrose throughout the whole period than did the fruit picked at the time of commercial

harvest. A comparison of the relative sucrose content and its fluctuation during ripening is shown in figure 4. The dextrose and levulose contents of fruit of the three pickings were about the same and differences in total sugars in fruit of the three pickings were caused by the differences in sucrose.

Starch might be expected to serve as the material from which sugars were formed during the ripening process. Microchemical tests, however, indicated that only slight traces of starch were present. Moreover, the alcohol insoluble residue which would have con-

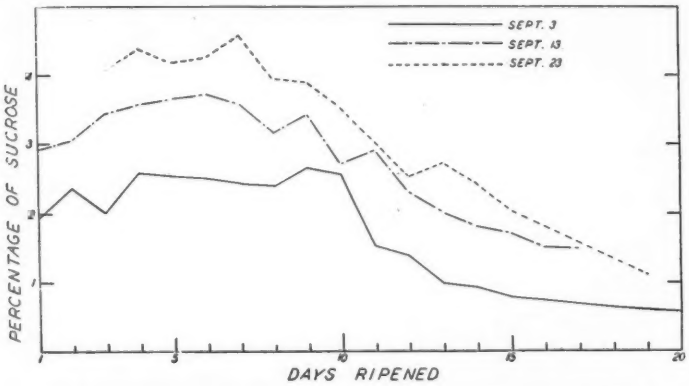


FIG. 4.—Effect of time of picking on sucrose changes during ripening.

tained the starch, if present, showed only very small decreases during the ripening of the fruit of series B and C, and only slight decreases in the fruit of series A. The magnitude of the observed decreases of this residue were found to be insufficient to account for the amounts of sugar observed to be formed during the ripening of the fruit. Starch determinations on the alcohol insoluble residues of the initial members of series A, B, and C showed 0.20, 0.20, and 0.28 per cent of starch respectively.

SORBITOL CHANGES IN RIPENING.—It was noticed from the data presented in the preceding section that the values of total sugars differed markedly from the corresponding values of soluble solids. This discrepancy was found to amount to 2.6–4.5 per cent of the fresh weight of the fruit, and suggested the presence of some major

constituent of a soluble non-sugar nature. The values of the differences between soluble solids and total sugars were calculated and designated non-sugar soluble solids. It was found that the values of this unknown undetermined constituent of the fruit decreased definitely during the ripening process and were roughly equal to or slightly in excess of the observed increases in total sugars. The values of this fraction of the fruit are shown in tables 5 and 6.

EMMETT (**5**) has reported that in the analysis of Conference pears there was a similar discrepancy between soluble solids and the sum of the separately determined constituents of the fruit. He calculated that 2 to 3 per cent of the fresh weight of the pear pulp was unidentified. Further, he noted that the amounts of this unidentified constituent decreased during storage, and suggested that the substance in question may have been sorbitol. He made no determinations, however, to substantiate this suggestion.

The presence of sorbitol in pears has been reported by VINCENT and DELACHANAL (**17**) and by REIF (**15**). Recently NUCCORINI and BARTOLI (**14**) have found that the sorbitol content of fruits of *Sorbus domestica* decreases when the fruit is detached from the tree, and they state that it is transformed into dextrose and levulose.

In view of these findings it was decided to determine qualitatively whether or not sorbitol occurred among the soluble solids of the Bosc pear. Certain of the alcohol extracts were evaporated to a thick syrup and this syrup allowed to react with benzaldehyde in the presence of sulphuric acid as a condensing agent. After standing overnight in the refrigerator, a white amorphous precipitate was obtained. This was then washed free of benzaldehyde and sulphuric acid. It was found to melt at 174°–177° C. and appeared to be dibenzal-sorbitol. DAVIS, SLATER, and SMITH (**4**) report that dibenzal-sorbitol prepared by this method melts at 179°–184° C. Owing to the difficulty of purifying this compound no recrystallization was carried out.

In order to demonstrate that the benzaldehyde derivative so obtained was definitely derived from sorbitol, a quantity of the material was hydrolyzed and then acetylated according to the methods given by TUTIN (**16**). The acetate so obtained was recrystallized from ethyl ether, and hard colorless crystals were ob-

tained. These crystals melted at 98°–99° C., which is the range reported by DAVIS, SLATER, and SMITH and by TUTIN for sorbitol hexaacetate. The acetate was also prepared by the same method from pure sorbitol; its melting point and appearance were found to be identical with that derived from pear extracts as just described.

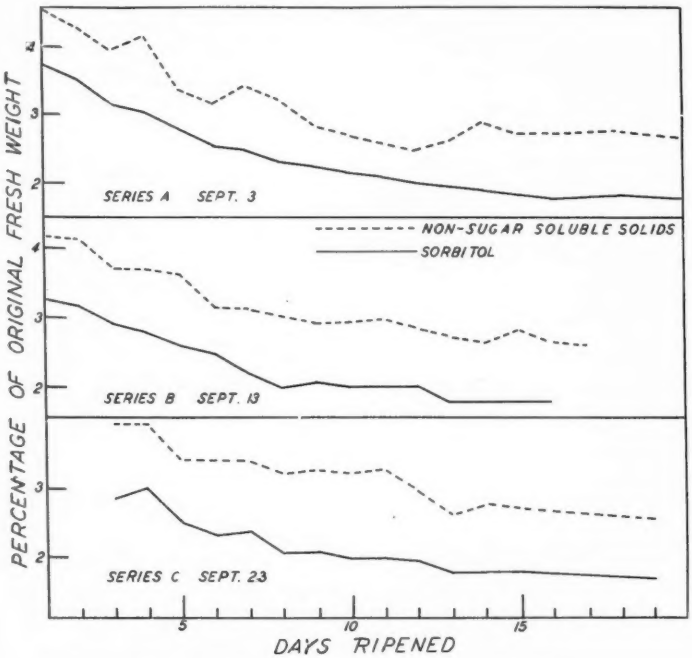


FIG. 5.—Relation of sorbitol to non-sugar soluble solids.

From the evidence presented it was assumed that sorbitol was definitely a constituent of the Bosc pear. Quantitative measurements were then made using the method outlined in the section on methods. The changes in sorbitol during ripening are listed in table 6 and shown graphically in figures 3 and 5. The changes in sorbitol (fig. 5) are plotted together with the corresponding changes in the

hitherto unidentified fraction which was designated as the non-sugar soluble solids.

The data shown in figure 5 indicate that sorbitol definitely decreases during the ripening process. In the fruit of all three pickings the decreases in the non-sugar soluble solids were accompanied by corresponding decreases in sorbitol. The most rapid decreases in sorbitol were found to take place in the first six to eight days, when both sucrose and levulose were observed to be formed from some hitherto unknown source.

The fact that the curves for sorbitol (fig. 5) follow the curves for non-sugar soluble solids indicates that the undetermined fraction contained sorbitol and that as sorbitol decreased the value of this fraction decreased. After subtracting sorbitol from the non-sugar soluble solids there still remained a value of 0.6 to 1.0 per cent, which seems reasonable for the sum of organic acids, soluble nitrogen, and other minor soluble constituents.

Discussion

In previous reports on the chemical changes in the ripening of pears, the duration of ripening has been taken as the time required to obtain fruit of good eating quality. In this investigation the results include data on the period following the attainment of optimum eating quality and continue to the time of occurrence of severe internal breakdown. The period studied, according to the usual criteria, might be said to include both ripening and over-ripening. In comparing the data reported here with those of other published reports, therefore, comparable data must be taken.

The ripening process, from the point of view of sugar changes, may be considered to consist of two definite stages: the first in which sucrose is formed, and the second in which sucrose is hydrolyzed with corresponding increases in dextrose and levulose. The attainment of fruit of good eating quality appears to occur in the four days following the beginning of hydrolysis of sucrose.

The essential differences in the sugar content of ripe and over-ripe Bosc pears appear to be the relative amounts of sucrose and reducing sugars. Total sugars show no decrease with over-ripening, but the

amounts of reducing sugars are far greater and the amounts of sucrose far less than in fruit of good eating quality. The composition of sound and over-ripe Conference pears in storage has been determined by EMMETT (5), and his results show higher values of sucrose in sound than in over-ripe fruit, together with larger amounts of reducing sugars in over-ripe than in sound fruit. The results of the present investigation are in agreement with his.

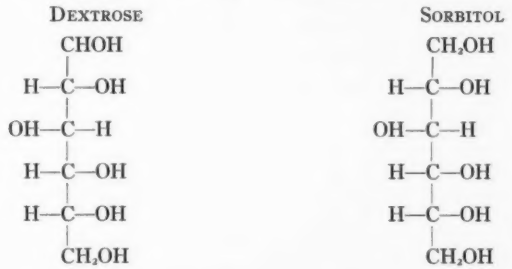
MAGNESS (12) and EZELL and DIEHL (6) have shown that in the ripening of Bartlett pears reducing sugars increased during ripening, and that in nearly all cases sucrose increased as well. If the composition of green Bosc pears is compared with that of ripe pears of good eating quality, essentially the same results may be observed, since during the first phase of ripening definite increases in the concentration of both sucrose and levulose were found.

MAGNESS has noted a much higher sugar content in fruit ripened at 70° F. than in comparable fruit ripened at lower temperatures. EMMETT (5), however, has suggested that the high sugar content observed by MAGNESS in fruit ripened at 70° F. may have been caused by a larger water loss by transpiration, for which no correction was made in the calculation of the sugar content. MAGNESS explained the larger increase in sugars at 70° F. as apparently due either to a smaller loss of sugar in respiration or to the fact that more insoluble or non-reducing substances in the green fruit are changed to soluble reducing material when the fruit is ripened at the optimum temperature.

The results presented in the preceding sections show the necessity of correcting for weight losses in ripening, but also indicate that even after such corrections are made, there are still definite increases in sugars which cannot be accounted for by decreases in the insoluble material of the fruit. The observed increases in sugars can easily be accounted for by the observed decreases in sorbitol. The presence of sorbitol in pears has been previously reported, but evidence for its transformation to sugars has not heretofore been presented.

Sorbitol may readily be oxidized to glucose chemically, and the manufacture of sorbitol is at present carried out by the reduction of glucose. It does not seem unlikely, therefore, that sorbitol could be oxidized to sugar in the processes of metabolism. The relationship

between the sugar alcohol, sorbitol, and dextrose may be seen from the following formulae of the two substances:



From the results presented here it appears that levulose and sucrose, rather than dextrose, are the sugars formed from sorbitol. During the most rapid decrease in sorbitol practically no dextrose was observed to be formed.

In the fruit of series B the transformation of sorbitol to sugars was almost quantitative, while in series A and C the decreases of sorbitol were considerably larger than the observed increases in sugars. In the latter two cases considerable losses in dry matter were noted, however, and it may be suggested that losses of sugar in respiration were sufficiently great that a smaller net gain in sugar was observed than would be expected from a quantitative oxidation of sorbitol to sugar.

NUCCORINI and BARTOLI have found that sorbitol is transformed to dextrose and levulose in the ripening of the fruits of *Sorbus domestica*. Such a transformation in the pear, accompanied by a preferential oxidation of dextrose in respiration, would lead to an excess of levulose over dextrose in the fruit. If the remaining dextrose combined with levulose to form sucrose and the excess levulose remained unchanged, we would observe in effect a transformation of sorbitol to sucrose and levulose, as appears to take place in the ripening of Bosc pears.

Whatever may be the mechanism of sorbitol metabolism in pears, it does definitely disappear in the ripening process. DAVIS, SLATER, and SMITH have found the heat of combustion of sorbitol in solution to be 732.44 kilogram calories per mol as compared with 676.08 for

dextrose. Thus an oxidation of a mol of sorbitol to glucose would release 66.36 kilogram calories of energy. The transformation of sorbitol to sugar by oxidation may therefore provide some of the energy for the metabolism of the fruit without loss of carbon dioxide, and thus represent a type of aerobic respiration which would not be recorded by the usual methods of measuring respiration.

Summary

1. A study of the ripening process of Bosc pears held at 67° F. has been made by means of chemical analyses of individual sample lots removed from the ripening room on each of 17–20 successive days. Pears of three different lots, picked ten days apart, were studied in this way and determinations made of total solids, alcohol insoluble residue, soluble solids, dextrose, levulose, sucrose, and sorbitol.

2. The losses of weight during the ripening period were recorded. The results of sugar and other determinations were calculated in terms of fresh weight at the time of sampling as well as in percentages of the original fresh weight. The latter method, involving correction for changes in effective size of sample taken, appears to give a more accurate method of studying the changes taking place during ripening.

3. A study of the sugar changes during ripening showed that levulose increased during the entire ripening period. Sucrose increased for the first six to eight days and then decreased throughout the rest of the period studied. Dextrose remained constant during the formation of sucrose, but increased later as sucrose disappeared. The increases in dextrose and levulose accompanying the decrease in sucrose were approximately equal, and their sum was about the same amount as that of the sucrose hydrolyzed.

4. The principal difference in the sugar content of fruit of the three pickings lay in the differences in the amounts of sucrose present. Throughout the ripening period the early picked fruit showed the least sucrose, the fruit of the commercial picking an intermediate amount, and the late picked fruit the highest values of this sugar.

5. Total sugars, calculated as the sum of dextrose, levulose, and sucrose, increased during the ripening process; this increase was

considerably greater than could be accounted for by the observed decreases in the alcohol insoluble residue.

6. Sorbitol was identified as a constituent of Bosc pears, and a quantitative method was devised for its measurement. Sorbitol was observed to decrease during the ripening process in fruit of all three pickings, and these decreases were large enough to account for the observed increases in total sugars.

The writer wishes to acknowledge his appreciation for the encouragement and helpful suggestions offered by Professors C. A. SHULL and S. V. EATON during the course of this investigation.

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DIFFERENTIATION OF THE SPIRAL VESSELS IN RICINUS COMMUNIS

FLORA MURRAY SCOTT

(WITH TWENTY-ONE FIGURES)

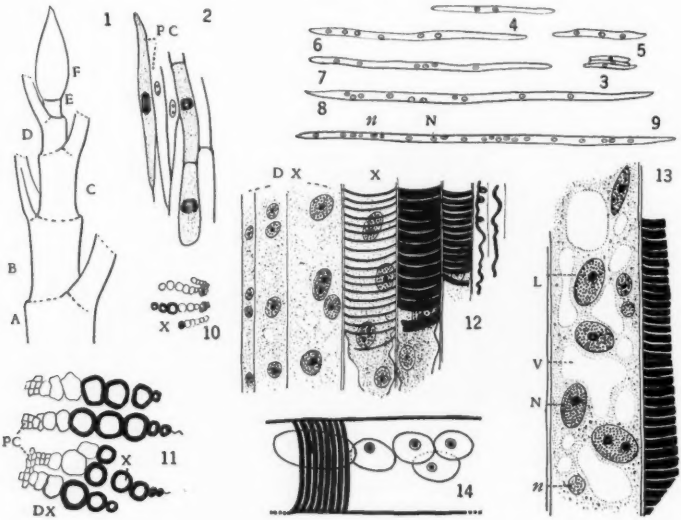
Although *Ricinus communis*, the Castor bean, is well known in botanical laboratories, the coenocytic development of the spiral vessels seems to have escaped attention. The Castor bean is perennial in California, forming a bush 6–8 feet high, which grows freely throughout the year.

The development of the spiral vessels may be followed in the primary tissues of the young vegetative or flowering shoots. Preliminary observations of fresh material were followed by a detailed examination of serial (imbedded) sections and by a study of macerated tissues. Unless sections are cut at least 12–15 μ thick, the contents of the developing spiral vessels as a rule escape in sectioning. In tangential sections 30–50 μ thick occasional coenocytes may be traced throughout the greater part of their length. The method of maceration proves most useful in this connection, for by it complete differentiating elements may be isolated for observation. By this method sections of the stem apex 30–60 μ thick, after treatment with 5 per cent chromic acid for two to four days, are stained in haematoxylin and mounted in glycerin. Tapping the coverglass with a needle is usually sufficient to separate the vascular elements. The tissues during maceration become extremely fragile, particularly the spiral vessels themselves, and a certain amount of confusion on the slide is therefore unavoidable.

In a typical dictyostele the region of elongation, composed of primary tissues, generally extends through nine internodes. The tip of the shoot, including the first five to six internodes, is inclosed by the characteristic deciduous bud sheath of the genus (fig. 1).

Growth of the shoot involves the usual processes of cell division and cell expansion (**6**). Microscopic examination reveals apical meristem, procambial cylinder, procambial strands, and ground tissue. The procambial strands differentiate into the vascular bundles of the

axis, leaves and bud sheaths. The first xylem elements to appear are small uninucleate spiral tracheids, two or three in number, at the base of the fourth leaf primordium. The largest spiral elements, in



FIGS. 1–14.—Fig. 1, diagram of young shoot in region of elongation. Lengths of internodes ($A-F$) and bud sheath: 6.5, 4.0, 2.4, 1.2, 0.6, and 3 cm. Fig. 2, longitudinal section of procambial tissue at base of leaf primordium; mitotic figures, anaphases, present in procambial element ($45\times4\ \mu$) and in two adjacent parenchyma cells. Figs. 3–8, typical coenocytes from various levels in first seven internodes of growing shoot (fig. 1) from macerated material: 3, 1-nucleate procambial element (198–216× 6 μ); 4, 2-nucleate coenocyte ($495\times7\ \mu$); 5, 3-nucleate ($405\times8\ \mu$); 6, 6-nucleate ($900\times12\ \mu$); 7, 7-nucleate ($1000\times13\ \mu$); 8, 10-nucleate ($1350\times16\ \mu$). Fig. 9, 19-nucleate coenocyte ($1475\times27\ \mu$) from living material, with two smaller possible degenerating nuclei. Fig. 10, transverse section of vessels of vascular bundle from internode *E*. Fig. 11, vessels of vascular bundle of internode *C;* diameter of lignified vessel, 70 μ. Fig. 12, longitudinal section, semidiagrammatic, showing adjacent developing coenocytes. Fig. 13, part of a living coenocyte (diameter 52 μ) showing vacuolated protoplasm. Fig. 14, detail of a group of nuclei within a vessel; length of large nucleus, 34 μ; of four small nuclei, 15–18 μ. *PC*, procambial tissue; *N*, nucleus; *n*, possible degenerate nucleus; *L*, nucleolus; *S*, spiral thickening; *V*, vacuole; *DX*, differentiating xylem; *X*, xylem.

the older internodes beyond the bud sheath, are multinucleate vessels whose maximum length is difficult to determine. Some may ex-

tend throughout the entire internode, their endings obscured in the tissues of the node. In any case the spiral thickening is continuous throughout the length of the vessel, and is uninterrupted by any trace of septa. The stages in the development of the coenocytic spiral vessels may be observed in the successive internodes of the region of elongation.

The first indication of differentiation of the xylem occurs in the procambial strands as they become defined below the meristem of the stem apex. Certain cells increase markedly in length and in diameter. Spiral thickening and lignification follow shortly, and result in the formation of uninucleate spiral tracheids at the base of the fourth leaf primordium already mentioned. Throughout the region of elongation a certain amount of cell division is going on constantly in vacuolating dividing cells—prophase, metaphase, and anaphase being most frequently noted (fig. 2). Cell wall formation does not always follow nuclear division, and binucleate coenocytes appear in the procambial strands (fig. 15). This coenocyte lies adjacent to spiral tracheids, horizontal in position, part of the vascular complex supplying the leaf base and bud sheath.

Where the differentiated vascular bundles begin to be established in the region of the fourth and fifth internodes, 3-nucleate and 4-nucleate coenocytes commonly appear in longitudinal sections, particularly where the leaf traces curve inward to join the main axis. Since as a rule the endings of these coenocytes pass out of the plane of the section, their length and complete nuclear number are seldom obtainable in sections, but they may be observed in macerated material (figs. 3–9). This series of diagrams illustrates typical stages in coenocytic development at different levels in the first seven internodes. A gradation in size and in nuclear number is apparent in passing from the stem apex downward. The measurements of the initial procambial elements and of the coenocytes illustrated are given in table 1.

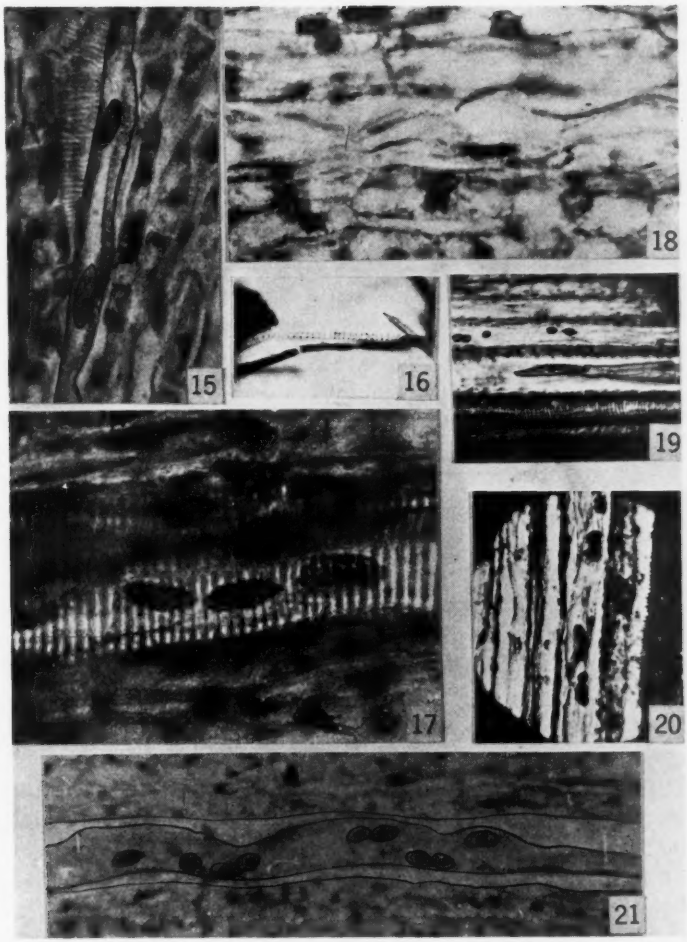
During primary growth the vascular bundles in successive internodes increase markedly in size of vessels and amount of lignification, although not in number of component elements seen in transverse section (figs. 10, 11). The vessels lie in radial series separated by xylem parenchyma. The final stages of differentiation of the larger

spiral vessels are generally best seen in longitudinal sections of the seventh or eighth internodes of the region of elongation (fig. 12). The youngest and least differentiated coenocytes, at the outer margin of the xylem strand, next to the future cambial zone, are narrow, thin walled vacuolating elements. A gradual increase in volume of lumen and of nuclei is seen in the older differentiating stages. The maximum expansion of the coenocyte is reached when its diameter slightly exceeds that of the adjacent lignified vessel. The protoplasm of the living coenocytes at this stage is highly vacuolated, the vacuoles varying much in size (fig. 13). Chondriosomes and granules are abundant, and the presence of small starch grains and a small

TABLE 1

	Length and width (μ)		Number of nuclei
Procambial cells	198–216	6	1
Coenocytes	495	7	2
	405	9	3
	900	12	6
	1000	13	7
	1350	16	10

amount of sugar is indicated by microchemical tests. Judging by the visibility of the nuclei, the protoplast may be in a moribund condition at the time of examination. The first trace of spiral thickening appears as a thin cellulose strand generally throughout the whole length of the coenocyte (fig. 16). It is less than 1 μ wide when first observed, and eventually widens into a close set, heavy band of cellulose, 2–5 μ deep. Examination in polarized light indicates that the cellulose micels are oriented approximately lengthwise in the spiral band. In transverse section the first traces of lignification appear at the cell corners and along the middle lamella, and may be demonstrated clearly by Maule's reagent, by phloroglucin and hydrochloric acid, or by Lauth's violet, an indicator stain. The spiral, when once lignified, continues to increase in thickness, but cellulose deposition and lignification now keep pace with each other. During this process the living protoplasm remains to all appearance unchanged. I was unable to observe any regular orientation of vacuoles such as has been described by Barkley (1), or any regularity



FIGS. 15–21.—Fig. 15, longitudinal tangential section through leaf base; binucleate coenocyte adjacent to differentiated spiral elements. Fig. 16, developing coenocyte in which cellulose spiral is formed (contracted during maceration). Fig. 17, longitudinal section; three nuclei visible within a differentiated spiral vessel. Fig. 18, longitudinal section; anaphase stage of mitosis in a coenocyte. Fig. 19, longitudinal section; contrasting sizes of nuclei obvious. Fig. 20, longitudinal section; close grouping of nuclei apparent. Fig. 21, longitudinal section; part of a 22-nucleate coenocyte. Paired arrangement of four nuclei seen to right.

in the arrangement of mitochondria or particles visible under ordinary conditions of illumination. While lignification is taking place, there is no visible disintegration of the living protoplast. The protoplasm and nuclei of the fully developed vessels remain visible in the lower internodes (fig. 17). The actual time of disintegration of the protoplasmic contents has not been determined.

TABLE 2

NUMBER AND SIZE OF NUCLEI OBSERVED IN INCOMPLETE COENOCYTES IN LONGITUDINAL SECTIONS

	Number of nuclei	Size of part of coenocyte observed (μ)		Size of elliptical nuclei: major and minor axes
		Length	Width	
Slide no. 6	2	90	8	14×3 27×6
	3	113	9	11×3 14×5 20×5
	3	90	10	14×4 15×4 23×5
	4	136	10	6×5 9×4 15×5
	4	150	12	7×4 7×4 14×5 14×5
Slide no. 15	5	150	14	7×4 8×4 11×5 11×5

It has already been stated that the spiral thickening as a rule appears simultaneously throughout the entire length of the coenocyte. In one preparation, however, one very thin spiral was observed to run only about three quarters the length of the element. The question, therefore, as to whether the spiral actually appears simultaneously, as in the majority of cases, or progressively from one end in its very early stage, as one preparation appeared to indicate, remains an open one.

A striking feature about the nuclei of the spiral vessels is their increase in volume (figs. 14, 19). A typical procambial nucleus in one of the younger internodes (fixed material) measures 4 μ in diameter and 33 cubic μ in volume. Some of the large nuclei in the seventh internode of the same shoot are elliptical in outline, with major and minor sizes 40 and 10 μ respectively. The calculated volume ($\frac{4}{3}\pi a^2 b$) 8373 μ amounts to a 253-fold increase in cubic content. In fixed and stained slides the artifact nuclei appear reticulate in structure, indicating a resting condition. Nucleoli, one, two, or more in number, are constantly present. They are not homogeneous throughout but generally possess a central vacuole of unknown consistency. In fixed material a clear area surrounds the nucleolus.

The increase in nuclear number appears to be due to mitotic division, for in all the material examined no trace of amitosis was observed. Mitotic figures are frequent in the youngest internodes. In the larger coenocytes direct evidence of mitosis is admittedly scanty, for only three figures, one metaphase and two anaphases, were actually found (fig. 18). The variation in nuclear size here, however, may be significant. Where larger and smaller nuclei occur, as they do in about half of the coenocytes examined, it is seen that some of the larger nuclei measure approximately twice the size of some of the smaller, a ratio which may point to intermittent nuclear division. Table 2 gives typical figures.

In fresh material the difference in nuclear size is again apparent, and here some of the larger nuclei are three or four times the size of the smaller. Thus in the longest coenocyte (2565 μ incomplete) examined, of the twenty-three nuclei, eighteen spherical and five elliptical, the measurements are as tabulated on the following page. The significance of the increase in volume of the nuclei will be considered later, in the general discussion. It is noted further that nuclei of the same size often occur in pairs close together in the coenocyte, a fact which may also point to recent division (fig. 21).

In certain coenocytes a few spherical masses of protoplasm occur which stain more or less deeply with haematoxylin. They are about the same size as the smaller nuclei, but they lack a nucleolus and are not always clearly defined in outline. It is possible that they may be

degenerating nuclei. Occasionally in both fresh and stained material the nuclei are clumped together in the coenocyte. This may be an artifact condition produced in the actual cutting (fig. 20).

		Diameter (μ)
Spherical	1 nucleus	5
	8 nuclei	7
	2 "	8
	4 "	9
	2 "	10
	1 nucleus	27*

		Length (μ)	Width (μ)
Elliptical	nucleus	12	9
	"	11	7
	"	14	5
	"	19	6
	"	22	11

* This nucleus contains a large vacuole-like area near the nucleolus and is considered abnormal.

Discussion

Certain points of interest arise in connection with this account of the development of the spiral vessel: (1) source of lignin; (2) development of the endodermis and entrance of solutes into the xylem vessels of the root; (3) time of lignification; and (4) hypertrophy of the nuclei.

First, the opinion has generally been held and has been stated recently by Weevers (**11**) that lignification is bound up with disintegration of protoplasm. That disintegration may accompany lignification is possible in certain cases, but the present observations show that the two processes are not necessarily interdependent.

Following from this, in the second place, an interesting point arises in connection with the development of the endodermis, a tissue of maximum importance in the actively absorbing root. Priestley and North (**6**) have suggested that the suberin of the Casparian strip is the result of the breakdown of the protoplasts of the developing vascular elements, and this idea has passed into current acceptance. A re-examination of root structure appears to be necessary in order to define the relative time of development of spiral thickening and of

disintegration of the protoplast. This will determine whether the process of lignification is essentially the same or fundamentally different in root and in shoot. In the same paper (6) the question is raised of the entry of solutes from a living cell into an empty xylem vessel. If the protoplast of the spiral vessel is virtually present after lignification, then the osmotic forces of living cells alone are functional in the absorptive region of the root, and the question of entry into the dead vessel automatically disappears.

Third, another question of interest is the time required for the development of the spiral vessel, in particular for the laying down and the lignification of the spiral. It is known that the dividing wall in an algal cell is laid down in from two to three hours (10). The development of the spiral vessels takes place in rapidly growing internodes. In sectioning material it is seen very soon, as has been emphasized by BARKLEY (1), that certain stages in the process of lignification are rarely seen. There is no difficulty whatever in finding either expanding vessels with unthickened cellulose walls, or fully lignified spiral vessels. The intermediate stages of semi-lignification, however, are few and far between. The process of wall thickening and of initial lignification is therefore presumably a relatively rapid process. Since a photosynthetic plant is normally periodic in its functioning, it is probable that the process of lignification is also periodic. Limited data indicate that most active lignification occurs between 1 A.M. and 5 A.M.

Fourth, the hypertrophy of the nuclei in the developing vessel is of direct interest to the cytologist. GUILLIERMOND (3) states that nuclei in higher plants range from 5 to 50 μ in diameter. The enormous size of the egg nuclei of the gymnosperms and the cycads is well known, of course, 500–600 being usual dimensions. CHAMBERLAIN found in *Dioon* a nucleus measuring $1475 \times 380\ \mu$. GUILLIERMOND quotes BUSCALIONI in regard to the size of the endosperm nuclei of *Lupinus* which measure $300 \times 500\ \mu$. But data in regard to the change in nuclear size within the same plant are meager. GOLDSTEIN (2) describes and figures hypertrophied nuclei in the xylem parenchyma and in the storage cells of a normal *Lilium* bulb. In this case the nuclei pass through a lobed condition and eventually fragment. ROSEN (9) describes the nuclei of the young epidermal cells

of *Hyacinthus* as elliptical in outline and approximately 15,000 cubic μ in volume. The older nuclei are spherical in form and have decreased to 5800 cubic μ or less than one-half the initial volume. It is stated as a general rule that nuclei decrease in size in differentiated tissues. KLEINEBERGER (4) interprets this decrease as a loss of physiological activity. If the latter statement is accepted, then the physiological activity of the differentiating xylem elements is presumably highly intensified.

The question naturally arises, is the hypertrophy due to an increase in karyolymph, in chromatin, or in both substances? The variations in nuclear size suggest possible polyploidy, but this can be determined only when sufficient numbers of mitotic figures are available. Since meristematic division is a periodic function in apical meristems, it is not unreasonable to suppose that a similar periodicity may obtain here, in which case at some happy hour in the twenty-four the various phases of mitosis will readily be available to the cytologist. He will then determine whether the nucleus is virtually polyploid, or whether perhaps the nuclear material resolves itself into units resembling the giant chromosomes of *Drosophila*.

Summary

1. The development of the spiral vessels in *Ricinus communis* may be observed in the young internodes of the growing shoot.

2. Multinucleate coenocytes differentiate from uninucleate procambial cells. These increase in size in successive internodes and may be observed in macerated material. The coenocytes range in length from 90 to at least 2500 μ, and the maximum number of nuclei observed is twenty-three.

3. As the developing vessel expands, the cell wall does not thicken measurably. When the maximum expansion is reached, a fine spiral of cellulose is laid down throughout the entire length of the vessel. This increases in thickness and begins to lignify when about 2 μ wide (in transverse section).

4. The development of the spiral and the initial lignification is presumably a rapid process, since the intermediate stages of semilignification are relatively rarely observed. Lignification is presumably a periodic process and may occur most actively in the early

morning. The lignified spiral continues to thicken, but at this stage cellulose deposition and lignification keep pace with each other.

5. The protoplasm of the developing vessel does not visibly disintegrate during lignification, so that this process is not dependent on any visible breakdown of the protoplasm. This fact raises a question as to the source of the suberin and lignin materials in the endodermis of the root, and also entails a reconsideration of the problem of the entrance of solutes from living cells into allegedly empty spiral elements of the root hair region of the root.

6. Whether the hypertrophy of the nucleus is due to an increase of chromatin material or of nuclear sap, or of both, remains undetermined.

7. The question of polyploidy or of giant chromosomes in the hypertrophied nucleus remains for future solution.

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MORPHOLOGY OF SOME AMERICAN SPECIES OF PSARONIUS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 482

NORMAN J. GILLETTE

(WITH TWENTY FIGURES)

Introduction

Investigations of the genus *Psaronius* Cotta have been numerous, as have been also the investigations of the three form genera *Caulopteris* Lindley and Hutton, *Ptychopteris* Corda, and *Megaphyton* Artis. As early as 1864, STENZEL (**25**) investigated certain structural aspects of various species of *Psaronius*. ZEILLER (**28**) studied the fossil flora of the Coal Basin and Permian of Autun and Épinac of France, and described several new species, together with an anatomical description of some. RUDOLPH (**23**) undertook a comparative investigation of certain species of the genus and some of the living Marattiaceae. In 1906 STENZEL (**26**) again published a report on the anatomy of certain species, together with a taxonomic review of the more common members of the genus.

Most of the investigations have been made on the European species, and relatively little has been done with the American material. In several instances specimens described as species of *Psaronius* (**3, 4, 5, 10, 11, 12**) were shown later to be either examples of other genera (**8**) or the descriptions were altogether inadequate for recognition (**17**). LESQUEREUX (**18**) described *Caulopteris giffordi* from Peoria County, Illinois. This specimen was also referred to as *Psaronius giffordi* by POTONIÉ (**22**), although no thorough investigation of its structure had been made. FARR (**7**) described in great detail a fern stem that was collected in Iowa, and appended to his description that of *P. borealis* by MACBRIDE (**19**) for portions of a stem from the same horizon. Although FARR did not state that his specimen belonged to the same species as MACBRIDE'S, the fact that he included in his description that of MACBRIDE indicates that perhaps he considered these two as belonging to the same species.

The roots of *Psaronius* occur commonly in many of the American coal balls (**2, 14, 15, 27**), but it is only rarely that any stem remains are found in this type of preservation. GRAHAM (**9**) and NOÉ (**20, 21**) both mentioned stems of *Psaronius*, but these were not well enough preserved to permit study. Poor preservation is not unusual in coal balls, and the stem size of this tree fern is large. HOSKINS (**16**), however, described an incomplete specimen of *Psaronius illinoensis* from "a calciferous petrifaction from the McLeansboro formation of Illinois." This specimen consisted of a narrow zone of roots and a few vascular strands of the stem axis. It was not possible to ascertain the nature of the stem in the living condition, nor could it be associated with any of the larger groups of *Psaronius*.

The several species have been separated into two main groups by HIRMER (**13**), Distichi and Polystichi, either on the basis of the number of rows of leaves they possessed or on the number of foliar bundles at the periphery of the stem in a given transverse section. A third group, the Incertae, includes those imperfectly known species of which a part or all of the transverse section of the stem is unknown. To the Psaronii distichi belongs the genus *Megaphyton;* to the other group *Caulopteris* and *Ptychopteris*. Some species of *Caulopteris* and *Ptychopteris* based on fragmentary specimens would belong to the Incertae if they showed any internal structure.

The Psaronii polystichi have been subdivided into the Verticillati and Spirales (**13**); the species in the former group had their leaves in whorls whereas those in the latter possessed spirally arranged leaves. The main characters used to differentiate the species possessing spirally arranged leaves have been the number of leaf trace bundles appearing at the periphery of any transverse section of the stem, the presence or absence of gum canals in the parenchyma (especially of the woody body), the presence or absence of lacunae in the cortical parenchyma of the roots as well as in the ground parenchyma of the stem axis, and the presence or absence and completeness or incompleteness of the sclerenchymatous sheath tissue in and around the woody cylinder.

The plant material described in this paper represents the silicified remains of Paleozoic tree ferns belonging to *Psaronius*. The specimens were collected about 25 years ago by Mr. VIRGINIUS CHASE of

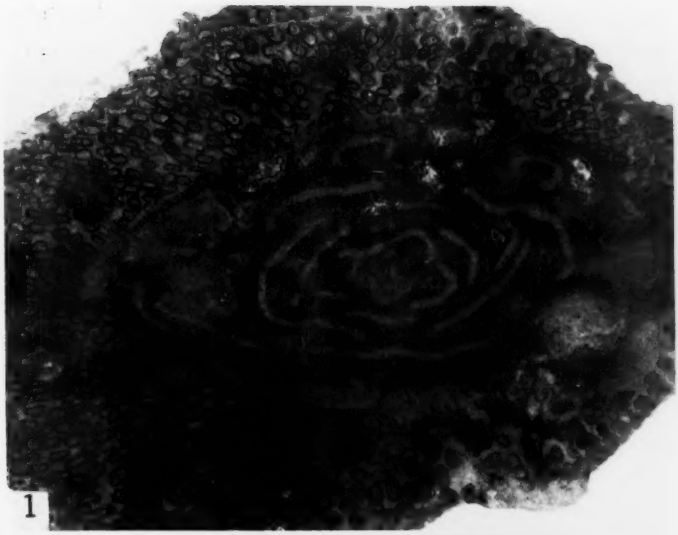
Peoria, Illinois, in a gravel pit in the vicinity of Peoria. This gravel pit has been abandoned long since. Its geological horizon is not known, but it was probably Pleistocene, and thus these fossils were not found in the strata in which they were originally preserved but were carried to that place by a glacier. Inasmuch as most of the European species of *Psaronius* are Permian, it is possible that these were also, although some species have been described from the Carboniferous. One specimen (no. 32) was collected by Mr. CHASE in Stark County, Illinois, and this may represent a Carboniferous species. The material was obtained from Mr. CHASE by Dr. A. C. NOÉ while the writer assisted him in field work for the Illinois State Geological Survey in the summer of 1935. The specimens are in the possession of the Peoria Academy of Science, and the number after each species refers to the accession number in that collection.

Since these stems were silicified and etching with hydrofluoric acid did not prove successful, the "peel method" of examining them was not possible. Thin sections were prepared, and the polished surface of the rocks examined. The model of the vascular skeleton of *P. septangulatus* Gillette (no. 2) was made from the study of a series of ten sections through a given stem, each section being about 1 cm. in thickness. The more detailed observations of the tissues were made on the thin sections.

Psaronius septangulatus sp. nov. (nos. 2, 5) Figs. 1–12

DIAGNOSIS.—In transverse section seven peripheral bundles alternate with seven foliar bundles. Within this zone several (17–20) strands are arranged more or less spirally. Definite sclerenchymatous sheath around woody body, interrupted opposite divergences of the leaf trace bundles. Ground parenchyma of stem axis dense with scattered gum canals. Leaf scars scattered, 2–2.5 cm. wide, 4–5 cm. high, elliptical, open at base and indefinitely closed at top. Vascular bundle scar large (2.5 cm. long, 1 cm. wide), horseshoe-shaped, in upper two-thirds of leaf scar. Leaves spirally arranged. Phyllotaxy 2/7.

Specimen no. 2 is a portion of a stem 27 cm. long, which in the living condition had a diameter of at least 11.5 cm., including the root zone, although in the state of preservation it is crushed so that its present diameters are 13 and 9 cm. The vascular portion of the



FIGS. 1, 2.—Fig. 1, transverse section of *P. septangulatus* (specimen no. 2) showing vascular cylinder surrounded by zone of adventitious roots. Fig. 2, surface view of *P. septangulatus* (no. 5) showing several leaf scars.

stem itself measures 9 by 5 cm. in diameter. There are few external distinguishing characters. The entire stem is covered with adventitious roots; no leaf scars are discernible. The vascular bundle scars of the leaf, as observations of the sectioned material show, are approximately 2.5 cm. long, 1 cm. wide, about 8.5 cm. from center to center on the same vertical row, and the vertical rows 3–3.5 cm. apart. The roots, somewhat intertwined around one another down the stem, give the surface of the stem a fluted appearance. The trees probably reached a height of over 10 meters (13), and the lower portion of the trunk was covered with a dense growth of adventitious roots.

Specimen no. 5 (fig. 2) is a flattened stem about 9 by 5.5 cm. in diameter and 19 cm. long. The petiolar scars, definitely marked on one side of the specimen, are separated from one another by about 2 cm. or less in the same vertical row and by about 2 cm. from side to side. The scar corresponding to the leaf bundle is elliptical, but the upper portion is open and its edges curve inwardly on themselves. The lower portion of the vascular scar is elevated above the surface of the rest of the leaf scar. The surface of the stem contains scattered pits which probably correspond to the divergences of the adventitious roots. These pits are especially prominent on the ridges between the leaf scars. Because of its external nature the specimen is associated with the genus *Caulopteris*.

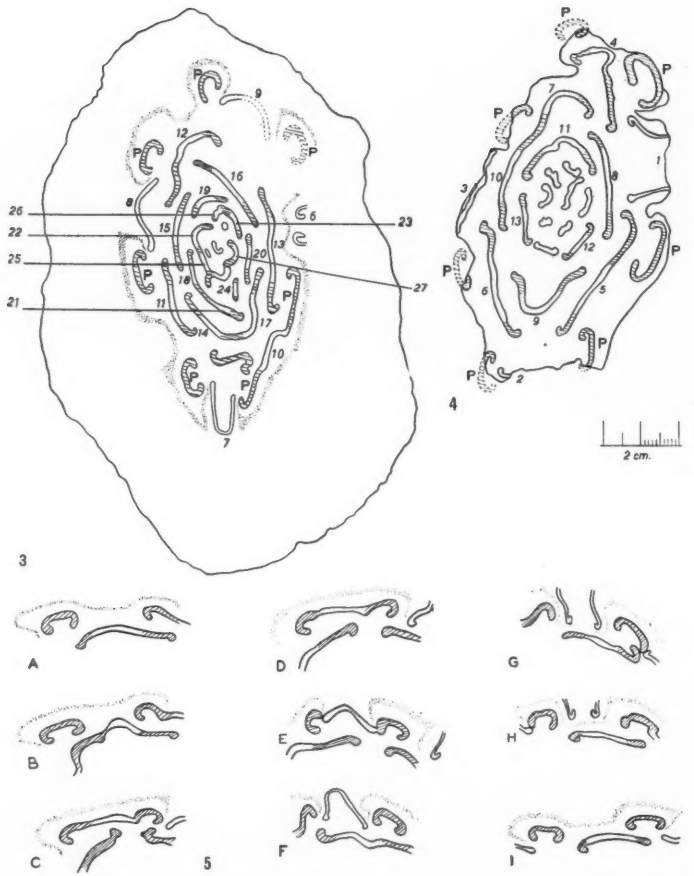
The peripheral bundles are rather strongly arched, with their edges curved inwardly. The tissue surrounding the xylem portion of the vascular strands is differentiated from that of the ground parenchyma. This was probably the phloem, although it is very poorly preserved, and it appears to encircle the xylem. The protoxylem is probably represented by groups of small cells on the inside of the vascular bands (fig. 12). Thus the stem would have been endarch. The parenchyma in which the vascular strands are imbedded is somewhat loosely arranged, although it is rather definitely not lacunar. Occasionally there are small groups of sclerenchymatous tissue near the periphery of the stem. There is no definite sheath, but if there had been one external to the peripheral bundles it probably would have been weathered away. Some of the parenchymatous cells are much larger than others, the larger ones con-

taining some deposited substance. These represent either tannin cells or the so-called gum canals. In general the cellular nature of the vascular tissue is better preserved in this specimen than in no. 2.

This species most closely resembles *P. spissus* Stenzel, but it may be distinguished from it by its greater number of peripheral bundles, five in *P. spissus* and seven in *P. septangulatus*. The woody body of the former species is somewhat smaller in diameter, and the peripheral bundles are separated from the ones on the inside by sclerenchymatous plates. This species differs from *P. demolei* Renault, *P. haidingeri* Stenzel, and *P. espargeollensis* Renault in the nonlacunar nature of the parenchyma as well as in the number of peripheral bundles.

VASCULAR ANATOMY.—(This description is based largely on observations made on specimen no. 2.) In transverse section (figs. 1, 3) the vascular portion of the stem shows a number of conducting strands imbedded in the ground parenchyma of the axis and which seem to be of at least three different types. Around the periphery of the vascular cylinder there are fourteen strands. These are alternately seven peripheral bundles (Randbündel of STENZEL) and seven foliar bundles (Blattbündel). At some levels, however, some of the peripheral bundles and foliar bundles are non-diverged from one another so that there may appear to be fewer than fourteen strands. Within this outer zone of vascular strands there are about sixteen other bundles (the Ersatzbündel and Innere Leitbündel of STENZEL). In transverse section these strands are elongate, platelike, more or less arched toward the center of the stem, and are arranged somewhat spirally in about five coils. Toward the center of the axis they become shorter in their transverse direction, and they are more closely associated. The small strands in the very center seem to have diverged from adjoining strands. In this way it is assumed that new leaf trace bundles may have arisen at the center of the stem. An examination of a series of transverse and longitudinal sections shows that the vascular system is constructed of a network of vascular strands anastomosing and diverging at various places (figs. 6, 7). The significance of these anastomoses and divergences is discussed in detail later.

The peripheral bundles are arched toward the center of the stem,



FIGS. 3–5.—Fig. 3, transverse section of *P. septangulatus* (specimen no. 2) showing vascular cylinder. Leaf trace bundles unlined and numbered 6–27; peripheral bundles (*P*) and other cauline bundles lined. Sclerenchymatous tissue stippled. Root zone outside of sheath. This drawing is from same transverse section as fig. 1 and represents level at top of schematic diagrams of figs. 6 and 7. Fig. 4, transverse section of *P. septangulatus* (no. 5) showing vascular strands. Peripheral bundles (*P*) and other cauline strands lined, foliar strands unlined. Bundles shown by broken lines represent reconstructions. Figs. 3 and 4 both drawn to same scale. Fig. 5, series of diagrams showing divergence of leaf trace from zone of steles just inside peripheral zone to base of petiole. Cauline strands lined, foliar parts unlined, sclerenchyma stippled.

and their lateral edges are strongly incurved. These measure about 8 to 15 mm. along the circumference of the stem. The part these bundles play in the formation of the foliar strands will be considered later.

Alternating with the seven peripheral strands are the foliar bundles. The edges of these latter may still be non-diverged from the

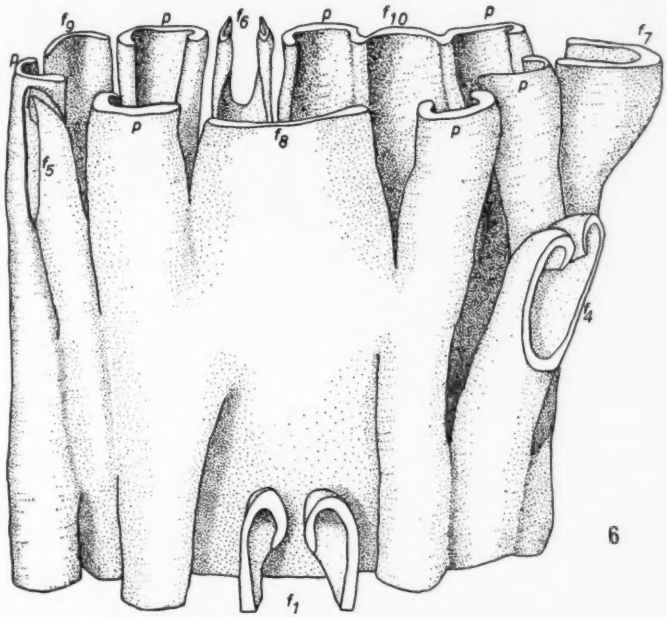


FIG. 6.—Schematic diagram of peripheral portion of vascular skeleton of *P. septangulatus* showing anastomoses of leaf traces with peripheral steles and subsequent divergence into bases of leaves. Peripheral bundles, *P;* foliar strands, f_1, f_4–f_{10}.

peripheral bundles in certain of the transverse sections. In the peripheral region of the woody body any foliar bundle may vary in form, from a plate parallel to the circumference of the stem, to a U-shaped strand with its free ends toward the stem center, to finally two apparently separate strands with the innermost edges curved toward each other, forming J-shaped strands (figs. 1, 3). The appearance of the foliar strands in transverse section depends on their

degree of divergence from the stem axis. The petiole bundle is horseshoe-shaped with its free edges curved inward (*cf. Stipitopteris*). Each foliar bundle forms an arc with a diameter of 8 to 15 mm., so that this measure corresponds to the width of the vascular

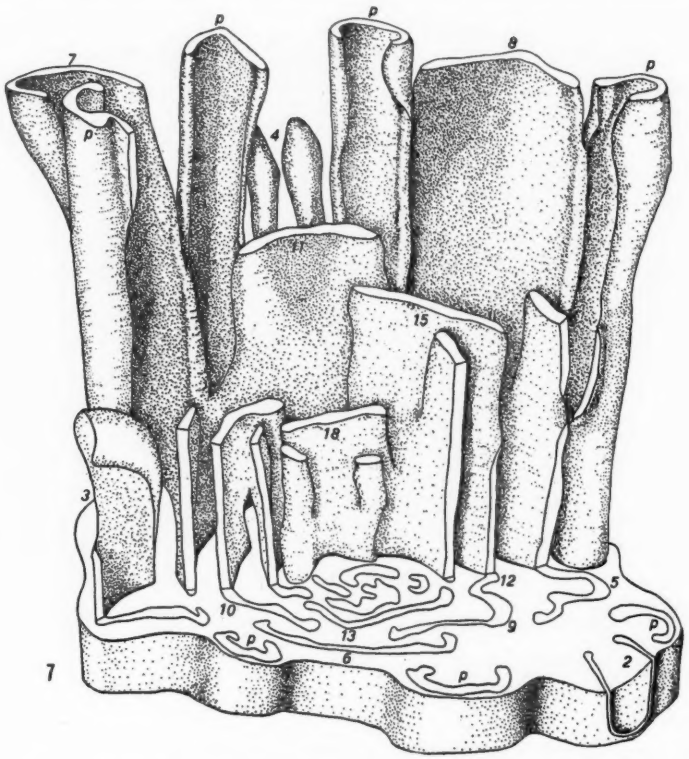


FIG. 7.—Same as fig. 6, but seen from inside. Vascular tissue dissected away from half of stem to show anastomoses and divergences of foliar strands with cauline strands as these former follow their course outward and upward. Peripheral bundles, *P;* foliar traces numbered with Arabic numerals.

bundle scar of the leaf scar, were it not obscured by the growth of adventitious roots on the outside of the stem. It is possible in this specimen to trace the foliar strands almost to the center of the stem axis. The final divergence of each strand is from the two adjoining

peripheral bundles, but it is clear that before this trace anastomosed with these peripheral strands it diverged from vascular plates lying adjacent to and inward from the peripheral bundles. In its divergence from the Innere Leitbündel (fig. 5) a gap is produced, so that in a section made at a higher level this strand with its gap has the appearance of two separate strands. The gap formed by this trace is made complete by the anastomosis with the leaf trace of the leaf immediately above this one leaf, as this subsequent leaf trace diverges outward from the next inner conducting strands. This formation of leaf gaps by the divergence of leaf traces from the conducting strands and the subsequent completion of these gaps by the anastomoses of the traces of the next higher leaves and the side flanks of the vascular strands continues as the leaf traces diverge outward and upward from near the center of the stem axis. Since there are about five coils in the spiral formed by the vascular bands of the woody body, there are five of these anastomoses and divergences before any given leaf trace bundle reaches the stem periphery. The side flanks of these Innere Leitbündel that do not diverge outward, together with the Randbündel, form the cauline system which continues up the stem.

In growth and maturation of the plant tissues, and especially of the vascular tissue, it seems probable that the center of the stem axis remained undifferentiated long after the more peripheral parts at that level were mature. Thus the undifferentiated and partially meristematic region of the stem axis was in the form of an inverted cone. In this way it is possible to explain the fact that the leaf bundles can be traced so far down the stem, as one follows them to the center of the woody cylinder. If the situation were as pictured, the new leaf trace bundles would arise probably as branches from the innermost complex of vascular strands (or provascular strands at the time of their origin). The angle which this leaf trace makes with the stem axis as it diverges outward and upward is approximately 7°. This means that the leaf trace originating at the center of the stem at any given level will diverge into the petiole approximately 27 cm. higher up the stem, since the diameter of the woody cylinder may be taken as 7 cm.

The origin of the foliar bundles in this species differs somewhat

from that described by ZEILLER (28) for *Psaronius infarctus* Unger var. *hippocrepicus*. According to him the foliar bands always leave the anastomosis of the vascular bands of the woody body nearest the periphery. This of course is as in the present species, but in the specimen examined by ZEILLER it was impossible for him to trace these strands further toward the center of the stem than just this peripheral region. In this case the leaf trace is described as consisting originally of two distinct halves which have their origins in the anastomoses of the peripheral bands, or a branch from these, with one of the bands at the edge of the central region. These two halves are then united into a single plate, the foliar bundle. STENZEL (26), on the other hand, described another variety of the same species, *P. infarctus* var. *quinquangulus*, in which the leaf trace bundles could be seen as portions of the conducting strands of the region just inside the peripheral circle of bundles; in fact it might be possible to follow a given trace even further toward the stem center. RUDOLPH (23) described a condition that supported STENZEL'S conception that the anastomoses appearing between the inner vascular strands probably represented the deeper parts of leaf traces. He described three types of vascular bundles in the stem: the foliar strands, other strands running vertically through the stem (these form a part of the cauline system as defined here), and the so-called peripheral bundles of ZEILLER. RUDOLPH described the course of the leaf trace strands as series of anastomoses and divergences from these cauline strands, in a manner similar to that here described.

HIRMER (13) described three types of leaf trace divergences in the Psaronii polystichi which possessed whorled leaves, the Verticillati. In the one type, for which he used *P. bibractensis* Renault as the example, the leaf trace is derived from the middle of a bundle plate, the side flanks of which form the peripheral bundles of the woody cylinder at the level of divergence of the leaf trace into the petioles. He described these peripheral bundles as ending blindly and disappearing at the edge of the stem. HIRMER thus pictured the vascular tissue as a series of funnels placed one inside the other, each cycle of leaf traces and their associated cauline bundles representing one funnel. In the second type, that of *P. infarctus* Unger, he found supplementary bundles that had no part in the formation

of the leaf trace. The foliar bundles in this type, however, arose in a manner very similar to that just described for *P. bibractensis*. There are thus alternating "secondary funnels" of vascular tissue which contain no part of the foliar system, but which are closely associated with it, and the "primary funnels" which contain the leaf traces. The third type is exemplified by *P. quadrangulus* Stenzel, and it behaves almost the reverse of *P. bibractensis*. In this case the foliar bundle is formed from the edges of the bundle plates, the central portion of each plate forming the peripheral cauline part of the system. Each leaf trace bundle in this case is therefore composed of two halves derived from as many bundle plates of the stem axis. These two halves unite in pairs at their edges and form together a single horseshoe-shaped leaf trace. In this type, as in the other two just described, the whole vascular system is in the form of funnels placed one inside the other.

FARR (7) described a species of *Psaronius* (*P. borealis* Macbride?) from the Upper Carboniferous of Hardin County, Iowa, which possessed eight longitudinal rows of leaves which he stated appeared to be spirally arranged. It appears from his figures, however, that the leaves were probably arranged on the stem in alternating whorls of four leaves each. The description given by FARR agrees only in the larger details with the specimen here described. He stated that it is apparent "that all leaf traces originate from a central strand and after more or less anastomosis proceed individually to their respective leaf bases. . . . . In their course they fuse laterally with the leaf traces of the whorl immediately above and that immediately below. In this way two concentric vascular rings are seen to be formed enveloping the central strand." Although FARR does not indicate that the cauline system played any role in the formation of the leaf trace strands, except perhaps a part of the central strand, he mentions that a "horse-shoe-shaped vascular strand extends from the base to the apex of the stem" between the rows of leaves. No mention is made of any sclerenchymatous sheath around the woody cylinder of the stem, nor is the nature of the ground parenchyma indicated.

All these species, *P. quadrangulus*, *P. infarctus*, *P. borealis*, and *P. bibractensis*, are members of the group of Psaronii which pos-

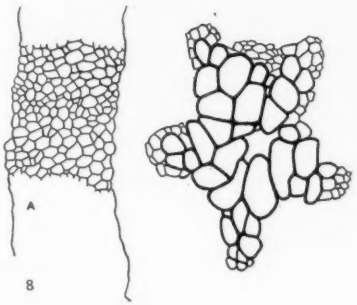
sessed whorled leaves. Little seems to be available concerning the anatomy of those species with spirally arranged leaves. HIRMER is of the opinion that in these species the vascular strands of the woody body are in the form of a "funnel-shaped winding staircase" with the various foliar traces diverging outward as well as upward in the stem. These vascular bands apparently represent the foliar strands as well as the cauline bundles from which these former are derived.

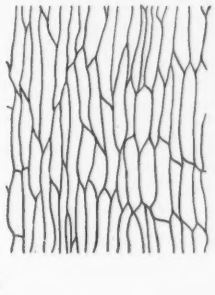
The vascular strands of the stem here described are composed mainly of xylem elements which are rather well preserved in some regions. Where their cellular details are not preserved, they have been infiltrated with a quartz-like material which appears as a contrasting color on the brown ground parenchyma. The xylem is composed of rather large scalariform tracheids which are relatively long with blunt tapering ends. Although no protoxylem has been identified in the longitudinal sections, it is assumed that these stems were either mesarch or endarch, and probably the latter. Around the xylem of the strand there is in some places a tissue that is differentiated from the ground parenchyma. In this region, where any cellular details can be seen they are very small elements with very thin walls. In all probability this represents the phloem region. It is not unusual that this is poorly preserved, because of the nature of the tissue in its living state. In some regions in the stem it is possible to see deposits in the cells of this region, which might represent accumulated food stuffs. This zone of cells seems completely to surround the xylem.

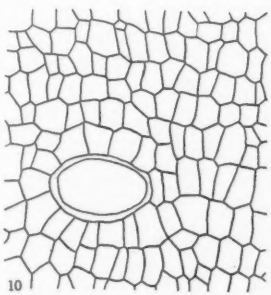
The ground parenchyma of the woody axis is non-lacunar. Its cells are relatively large, thin walled, and isodiametric. Some of the cells contain a deposit of opaque material which may be tannin or some other accumulated plant product. Although there are no lacunae, the cells are not so compact that they form sharp angles in their corners; the reverse is the case, for the cells seem so loosely arranged that each is nearly spherical. These cells vary only slightly in size.

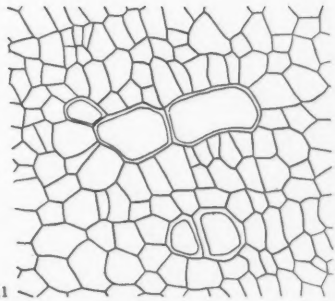
Surrounding the central vascular region is a sheath of sclerenchymatous cells. These are much smaller than either the ground parenchyma of the axis or the xylem tracheids (figs. 8*A*, 9). Its cells

are compact, somewhat angular, and have thick walls. In longitudinal section they are somewhat elongate and tapering but not nearly so long as are the tracheids. The cells of this sheath are not clearly marked off, either from those within or from those immediately out-









FIGS. 8–11.—Fig. 8, stele of pentarch root of *P. septangulatus* together with some sclerenchymatous tissue of outer cortex of root (*A*). Fig. 9, longitudinal section of some of cells from sclerenchymatous sheath of *P. septangulatus*. Fig. 10, transverse section of parenchymatous tissue found outside of some of peripheral strands and in leaf scars of *P. septangulatus*. Tissue has appearance of a periderm, and in it are found numerous gum canals. One is shown here. Fig. 11, longitudinal section of same tissue as shown in fig. 8. All figures drawn to same scale.

side it. The sheath is not continuous in the region of the divergences of the leaf trace bundles from the axis.

The roots that surround the woody cylinder are numerous and

pass down the stem in somewhat vertical rows, although there is some intertwining. The steles of these roots are pentarch or hexarch. The xylem in some is rather well preserved, with the large metaxylem in the center and the protoxylem toward the outside (fig. 8). The phloem and the inner cortical parenchyma are not well preserved, but in those regions where some of the inner tissue is preserved it appears to be non-lacunar. The outer region of the root is of thick walled sclerenchymatous cells. These are of the same type as are the cells of the stem sheath; in fact the tissue of the sheath of these roots first appears associated with the steles in the sclerenchymatous sheath of the stem. The vascular elements of the root are derived mainly from the peripheral bundles of the stem, although some may be seen diverging from leaf trace bundles within the peripheral zone. In the root region of some species it is possible to differentiate two separate zones, an inner one in which the roots are imbedded in relatively dense parenchyma, and an outer zone where the roots are more or less free from one another. This specimen shows only one zone of root development.

The parenchyma outside the sclerenchymatous sheath of the roots, in which these are imbedded, consists of somewhat elongate cells in numerous closely intermingled multicellular filaments, having the appearance of a homogeneous tissue; but the filaments seem "combed" in various directions. Concerning the origin of this parenchymatous tissue there have been two main ideas. STENZEL (**25**, **26**) supposed that the inner portion of the zone was a part of the cortex of the stem itself, the roots being intrusive structures. RUDOLPH (**23**) likewise thought that the regular arrangement of the roots showed that they penetrated a tissue already formed. FARMER and HILL (**6**) have compared the root zone of the present day Marattiaceae with that of *Psaronius* and found that the root is an intrusive organ in the former, whereas in *Psaronius* the outer cortical region of small-celled sclerenchyma of the root passes almost insensibly into the parenchymatous tissue between them. SOLMS-LAUBACH (**24**) has shown that the filling-in tissue might have originated from a cellular proliferation at the stem periphery as well as at the root periphery. BERTRAND (**1**) likewise concluded that this filling-in tissue was "due to a proliferation of a suberose nature, produced at the expense of the superficial tissues."

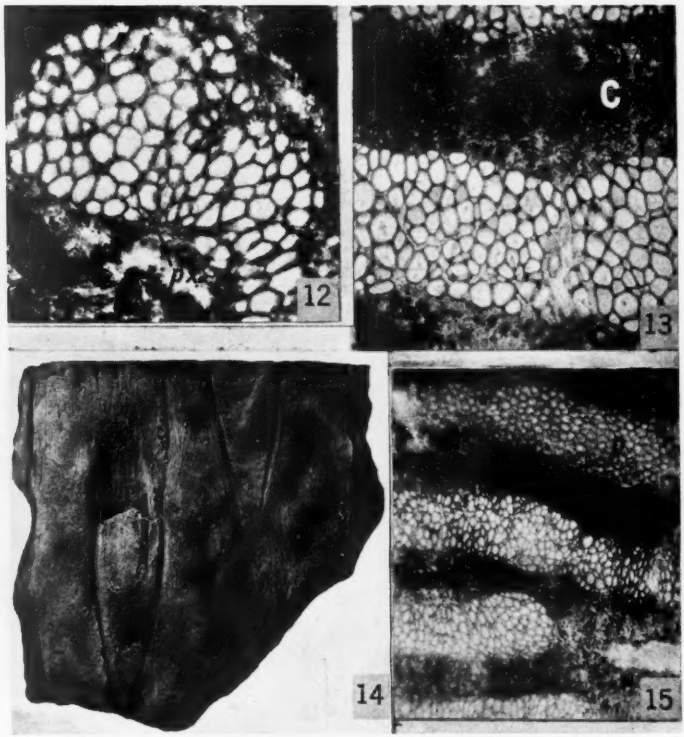
The cells just inside the root zone are differentiated like a periderm (figs. 10, 11). This type of tissue is found especially in the region of a leaf base, but may also be seen outside some of the peripheral bundles. It would appear that in the former case it was formed at the time of leaf fall, or just subsequent to it. It was probably developing at the time the roots were descending on the stem outside it, and by means of this periderm-like growth the irregularities in the stem circumference due to grooves, ribs, etc. were smoothed out by the time the roots were developed at that level. Since the cells in this zone have such a definite orientation, it is possible to detect definite gum canals which could not be identified with certainty in the more loosely arranged ground parenchyma of the woody axis.

Psaronius peoriensis sp. nov. (no. 3) Figs. 14–16

DIAGNOSIS.—In transverse section seven peripheral strands alternate with seven foliar strands; within the peripheral zone about forty vascular strands. No definite sclerenchymatous sheath, but regions of sclerenchymatous tissue associated with the outer vascular strands. Parenchyma of woody body somewhat loose but not lacunar; gum canals scattered and few in number. Leaves rather separated; leaf scars subcontinuous, elongate, tapering at both ends, 10 cm. long, 2 cm. wide. Vascular bundle scar centrally located in leaf scar. Leaves spirally arranged. Phyllotaxy 2/7.

Specimen no. 3 is a flattened stem fragment 8 by 4 cm. in diameter and about 10 cm. long. The scars of the petioles on the external surface are arranged in approximately longitudinal rows. The scars corresponding to the vascular bundles are elliptical, 10–14 mm. wide and approximately 4 cm. long. These are probably open at their upper end with their free ends curved inwardly. The vascular scar is located about in the center of the leaf scar. The scars in the same vertical row are almost joined end to end, the rows themselves being 4 cm. apart from axis to axis. The surface of the stem between the scars is not smooth, but is covered with somewhat elongated but only slightly elevated warts. The verrucose nature can also be seen in some of the petiole regions where the outer portions of the scars have been destroyed. The bases of the adventi-

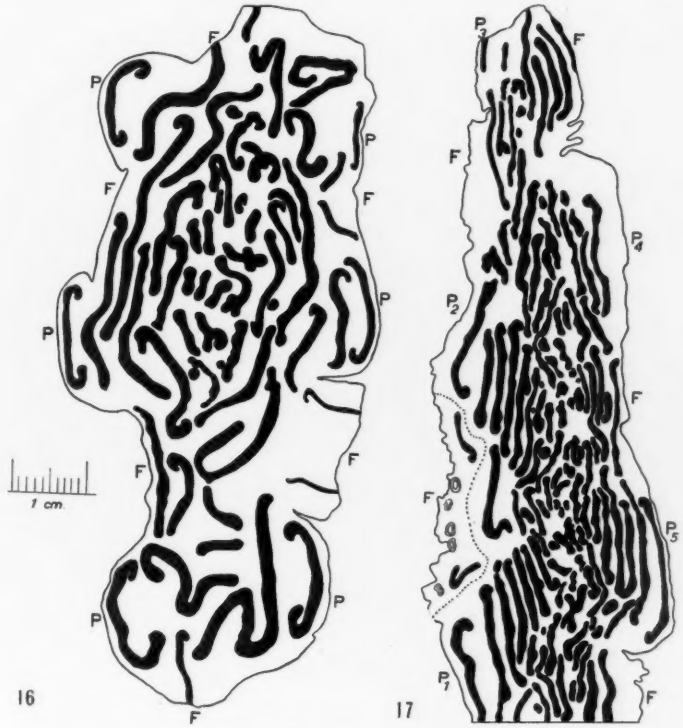
tious roots can be seen in various scattered places, especially in the regions outside the leaf scars.



FIGS. 12–15.—Fig. 12, transverse section of portion of vascular strand of *P. septangulatus* (specimen no. 5) showing protoxylem (*px*); endarch condition. Fig. 13, transverse section of portions of vascular strands of *P. giffordi* showing xylem tracheids. Some tannin cells (?) or gum canals appear as large dark round cells (*C*). Fig. 14, surface view of *P. peoriensis* (specimen no. 3). Fig. 15, transverse section of portion of stem of *P. peoriensis* showing portions of three vascular strands and some of ground parenchyma of axis (*P*).

In transverse section (fig. 16) there are clearly six peripheral bundles, but there were probably seven of these bundles alternating with the seven foliar strands, since the leaves were definitely not

whorled, nor did they have a phyllotaxy of 1/6. The vascular strands within the peripheral zone vary greatly in their transverse direction from circular strands to long bandlike strands. The foliar bundles do not seem to be very closely associated with the periph-



FIGS. 16, 17.—Fig. 16, transverse section of *P. peoriensis*. Peripheral bundles, *P* leaf traces, *F*. Fig. 17, transverse section of *P. giffordi*. Peripheral bundles, P_1–P_5. Both figures drawn to same scale.

eral strands in their divergence from the woody body; at least in the one transverse section there was no non-divergence of foliar strands from the peripheral strands as in most of the sections of the other species. The protoxylem of the vascular strands appears to be located on the inside of the curved vascular strands, and

in transverse section appears as groups of small cells. On the outside of these vascular bands in some places cells have been identified as phloem cells. These are not well preserved, however, and they are not present on the outer surface of all the vascular bands, although the tissue in that region has a different appearance from that of the ground parenchyma in those where the phloem is not preserved. This region occupied by the phloem is very narrow in comparison to that occupied by the xylem. The larger cells of this region and some of the cells of the ground parenchyma of the woody body appear to be filled with a dense black substance. This may be tannin or some other substance deposited in these cells.

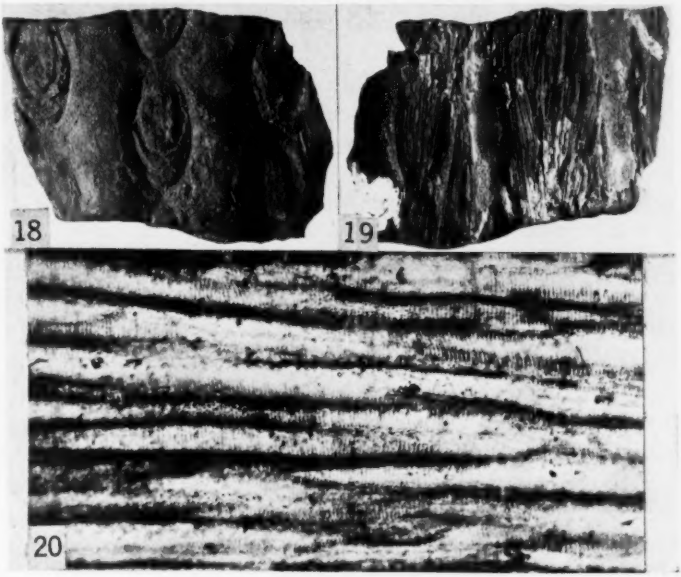
Psaronius giffordi Potonié (no. 32) Figs. 13, 17–20

DIAGNOSIS.—In transverse section six peripheral bundles alternate with six foliar bundles. Other strands very numerous, approximately 100, arranged more or less in six groups opposite the six peripheral bundles. Parenchyma. . . . . Numerous very large cells which may be gum canals. Leaf scars elliptical, open at the top as a horseshoe, 2–2.5 cm. wide, 4 cm. long, subcontinuous in the vertical rows. Vascular bundle scar in upper two-thirds of the leaf scar, horseshoe-shaped. Leaves in alternating whorls of three leaves each.

This specimen is the crushed portion of a stem showing in all nine leaf scars (which are arranged in six vertical rows) the members of alternating rows apparently forming the various whorls of leaves. Each whorl thus contained three members. The rows of leaf scars are about 4.5 cm. apart from center to center. The scars themselves are less than 1 cm. apart in any vertical row. The vascular bundle scars measure 14 mm. in width and 25 mm. in length. The tissue outside the petiolar scars contains sparsely separated pits corresponding to the divergences of the small adventitious roots. In addition to these larger pits the surface of the stem is finely grained. On one side of the specimen the root zone is still partially intact, especially over the rows of leaf scars. In this region it is possible to see the small adventitious roots imbedded in their matrix. These roots do not run parallel down the stem but are intertwined about one another.

In transverse section the numerous vascular strands are so closely

associated that it is difficult to determine anything about the origin of the leaf traces, etc. (fig. 17). The peripheral bundles around the edge of the stem, however, do alternate with the foliar traces as in the other species described. In general there seems to be more regularity of arrangement of the vascular strands opposite the



FIGS. 18–20.—Figs. 18, 19, views of the two surfaces of *P. giffordi* showing leaf scars. In fig. 19 the root mantle covers the leaf scars. Fig 20, longitudinal section of xylem tracheids of *P. giffordi* showing scalariform thickenings on walls.

peripheral bundles than opposite the foliar bundles. There thus appears in the stem in groups radiating from the center toward several of the peripheral bundles at least four definite groups at P_1, P_2, P_4, and P_5. Because of the crushed nature of the stem this group is not evident opposite P_3. The portion of the stem including P_6 is not present in this specimen, but it is assumed that there are six peripheral strands because of the apparent location of the center of the stem axis, and because of the whorled arrangement of the leaves.

The general construction of the woody body of this species may

have been similar to that described by HIRMER (13) for *P. infarctus*, in which there were present parallel to the bundle plates giving rise to the leaf traces "secondary bundle funnels" which had nothing to do with the emission of the leaf trace. Because of the nature of the preservation of this specimen it is not possible to determine anything concerning the origin of the leaf traces, as already pointed out. *P. infarctus* was a much larger stem, however, there being five to seven leaves in each whorl, so that there were ten to fourteen peripheral bundles.

The cellular nature of the xylem portion of the vascular strands is especially well preserved (figs. 13, 20). It is composed of relatively large tracheids which have scalariform markings on their longitudinal walls. The woody cylinder was surrounded by a rather indefinite sclerenchymatous sheath, but this had been destroyed in some regions of the stem. In the longitudinal section of the stem cut at right angles to the long diameter of the crushed stem, the vascular strands can be traced the whole length (6 cm.) of the section without anastomosis or branching. The ground parenchyma in which the vascular strands are imbedded is much crushed and poorly preserved, but it appears to have been of a dense nature. In some places, however, there are large spherical cells that seem to contain some sort of deposition substance. These may be what some interpreted as gum canals, or on the other hand they may represent tannin cells.

The external character of this specimen agrees rather closely with that of *Caulopteris giffordi* Lesquereux (18), except for the larger size of the scars of this latter. Inasmuch as no anatomical study has ever been made of *C. giffordi*, the correlation between the internal structures is not possible. POTONIÉ (22) called the internal portion of *C. giffordi Psaronius giffordi*. The specimen here described has been compared with the type specimen of LESQUEREUX'S species, specimen no. 41006 in the paleobotanical collection in Walker Museum.

Summary

1. A morphological study of four specimens of the Paleozoic tree fern *Psaronius* was made. These specimens were placed in the following three species: *Psaronius septangulatus* Gillette, *P. peoriensis*

Gillette, and *P. giffordi* Potonié. *P. septangulatus* and *P. peoriensis* possessed seven rows of leaves arranged spirally; *P. giffordi* possessed six rows of leaves arranged in two whorls.

2. The vascular anatomy of one of the specimens of *Psaronius septangulatus* was studied in detail from a series of transverse sections of the stem. The leaf traces diverge outward from the center of the stem axis in a manner somewhat similar to that described for *P. infarctus*, which possessed whorled leaves.

3. Thin sections of all three species were examined and described for the different tissue types.

The writer is indebted to Mr. VIRGINIUS CHASE of the Peoria Academy of Science for the material used in this study. Thanks are due also to Dr. A. C. NOÉ for helpful suggestions and criticism during the progress of the work.

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MEIOTIC STUDIES IN TRIPLOID TULIPA WITH SPECIAL REFERENCE TO BRIDGING AND FRAGMENTATION

MARK W. WOODS

(WITH THIRTY-ONE FIGURES)

Introduction

NEWTON (3) and NEWTON and DARLINGTON (4) have called attention to the autopolyploid nature of a number of tulips, and the meiotic behavior in three triploid garden tulips has been described in detail by these investigators (4). The present paper reports studies of microsporogenesis in the triploid cottage tulip, Inglescombe Yellow. Certain types of behavior, not hitherto described for *Tulipa*, have been determined in this variety. Special attention has been given to the formation and behavior of chromosome fragments, and to the distribution of chromatin bodies in interphases I and II. No attempt will be made to review here the extensive literature on chromosome fragmentation, and the occurrence of chromosome fragments in plants. MACMAHON (2) has recently published an excellent review of this literature.

Material and methods

Anthers were obtained in the fall from bulbs that had been grown one or more seasons in Maryland. Material to be sectioned in paraffin was fixed in Flemming's medium solution or Navashin's fluid. Heidenhain's iron-alum haematoxylin was used to stain the material. Most of the studies were made from temporary smears prepared by the aceto-carmine technique, but permanent smears were also made according to the method of TAYLOR (6). Observations were made with a 90× apochromatic oil immersion objective of n.a. 1.30 and a 15× compensating ocular. All drawings were done with the aid of a camera lucida.

Results

Chromosome behavior in microsporogenesis

Diakinesis and metaphase I.—Trivalents, bivalents, and univalents occurred in prophase I and metaphase I. Table 1 gives the results of a detailed examination of 53 pollen mother cells in which every chromosome association was determined. The average number of trivalents per pollen mother cell was 9.58, or approximately 80 per cent of a complete trivalent association. Some of the observed

TABLE 1

Chromosome associations at late diakinesis and metaphase I in Inglescombe Yellow (3n equal 36). Data for 53 pollen mother cells

Total number of chromosome associations per pollen mother cell				
Trivalent	Bivalent	Univalent	Observed frequency	Percentage of total number observed
5	7	7	2	3.77
6	6	6	0	0.00
7	5	5	4	7.54
8	4	4	6	11.32
9	3	3	7	13.30
10	2	2	19	35.83
11	1	1	11	20.75
12	0	0	4	7.54

trivalent configurations are shown in figures 1–28. In most of the trivalents only two of the homologues were associated at a single point (figs. 3, 6, 13). Sometimes three homologues were closely associated in the region of their kinetochores (spindle attachment regions), although the actual unions may not have occurred between more than two of the chromosomes at a single locus (figs. 1, 5).

Associations like those shown in figures 27 and 28 were rarely observed. In such cases distinct repulsion had occurred between the completely associated homologues and the third chromosomes at both ends, but all three were associated in the middle.

Anaphase I.—By late metaphase or early anaphase each homologue was split into two daughter chromosomes. Various irregulari-

FIGS. 1–28.—Trivalent configurations from late diakinesis and metaphase I of microsporogenesis in Inglescombe Yellow. Figs. 5 and 10 from diakinesis; remainder from metaphase.

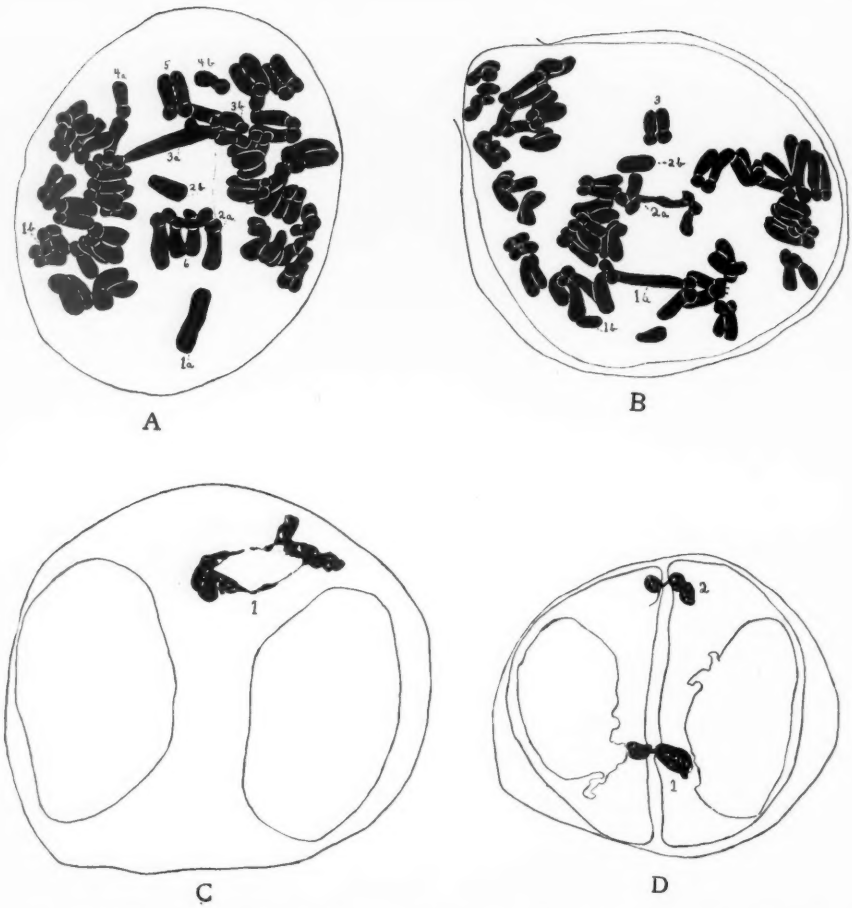

FIG. 29.—Microsporogenesis in Inglescombe Yellow. *A:* anaphase I with three cases of fragmentation and associated irregularities. *1a* is kinetochoreless fragment deleted from two daughter chromosomes at *1b*. Whole configuration (*1b*) passed to one pole instead of forming a bridge. A typical bridge with its accompanying fragment is shown at *2a* and *2b*. Long bridge at *3a* is accompanied by very short fragment attached to distal end of daughter chromosome *3b*. *4a* and *4b* are daughter chromosomes derived from single univalent. 5 and 6 are lagging, but divided univalents. *B:* anaphase I with two cases of bridging (*1a* and *2a*) and their accompanying fragments *1b* and *2b*. Note that fragment *1b* is attached to distal end of one of daughter chromosomes. *C:* large mass of chromatin excluded from daughter nuclei in first division. *D:* excluded chromatin fragments apparently being cut in two by developing cell plate.

ties occurred during anaphase. The bivalents most often separated in a regular fashion with one pair of daughter chromosomes going to each pole. The trivalents and univalents, however, frequently behaved irregularly (fig. 29*A*). Table 2 presents an analysis of ten ana-

TABLE 2

CHROMOSOME DISTRIBUTIONS IN TEN POLLEN MOTHER CELLS OF INGLESCOMBE YELLOW AT ANAPHASE I

No. of P.M.C.	Daughter chromosome pairs (pole A)	Single chromosomes (pole A)	Daughter chromosome pairs (pole B)	Single chromosomes (pole B)	Lagging undivided univalents	Lagging divided univalents	Other lagging chromosomes
1.....	17	1	15	1	0	0	1 bivalent
2.....	16	0	16	1	1	2	.....
3.....	16	0	16	0	0	4	.....
4.....	17	2	17	0	0	0	.....
5.....	14 irreg.?	1	17	2 plus a fragment	1	1	.....
6.....	14	0	16	1	1	3	1 bivalent
7.....	18	0	17	0	1	0	.....
8.....	15	0	18	0	1	0	1 bivalent
9.....	14 plus a fragment	0	19	1 fragment	0	2	.....
10.....	17	2	14	0	0	3	.....
Average	15.8	0.60	16.5	0.50	0.50	1.5	0.30 bivalent

phase I chromosome distributions. From this and other data, it has been determined that the following events may occur at anaphase I:

1. One or more of the univalents may fail to divide, and then lag or go to one of the poles.

2. The univalents may divide, after which they may lag, or one daughter chromosome may go to each pole; or both daughter chromosomes may go to the same pole.

3. Bivalents may lag.

4. Various irregular configurations involving bivalents or trivalents may occur. These sometimes form bridges or exhibit other irregular behavior. Fragment chromosomes result in certain cases.

Chromosome bridging and fragmentation have been studied in particular. Typical cases of bridging are illustrated in figure 29*A*, *B*. Figure 30*A* is a photograph of the cell shown in figure 29*A*.

Bridging and chromosome fragmentation are closely associated. A schematic interpretation is given in figure 31. The diagrams in this figure are lettered and numbered to correspond with the actual drawings, which appear in solid black. Metaphase I trivalent configurations like the one shown in figure 31*A* (an enlargement of figure 28) have been observed. The opening out of such a configuration in anaphase could conceivably result in the situation shown in figure 31*B*, *C*, *D*. Detailed analysis of a number of cases of simple bridging and fragmentation have shown (*a*) that the length of its

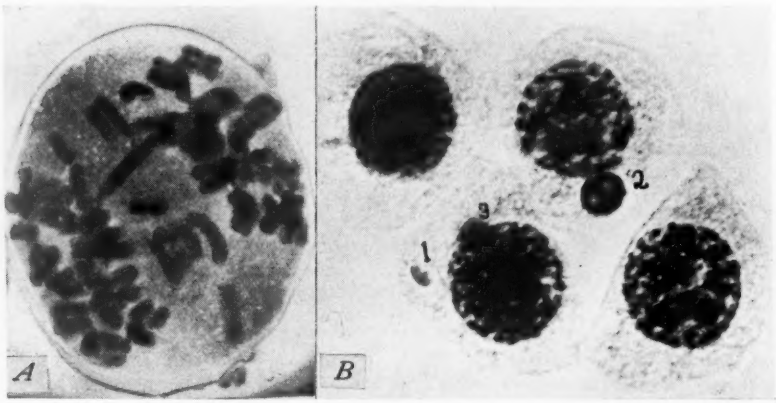

FIG. 30.—Microsporogenesis in Inglescombe Yellow. *A:* anaphase I with three cases of fragmentation and associated chromosomal irregularities (same cell as shown in fig. 29*A*). *B:* tetrad of microspores showing extra nuclear chromatin bodies at points numbered 1, 2, 3.

bridge (fig. 31*C*) is inversely proportional to the length of the fragment lacking a kinetochore (fig. 31*D*); (*b*) that the bridge always has two kinetochores being formed from portions of two chromosomes distally united; and (*c*) that the number of kinetochoreless fragments is equal to the number of bridges or similar configurations. In some cases the fragment is attached to the distal end of one of the daughter chromosomes that is not involved in the bridge (1*b* of fig. 29*B*). Unless attached to another chromosome in this manner, the fragment fails to move in anaphase. The fragments lacking kinetochores have not been observed in a divided condition, although there was very slight evidence of a split in chromosome 1*a* of figure 29*A*.

A situation like that illustrated in figure 29*A* (chromosome groups 1*a* and 1*b*) should result in bridging in the second anaphase. In this instance the breaks in the two homologues occurred very close to the kinetochores; but instead of forming a bridge in anaphase I the whole kinetochore-bearing configuration (1*b*) passed to one pole, leaving the fragment (1*a*) at the equatorial plate.

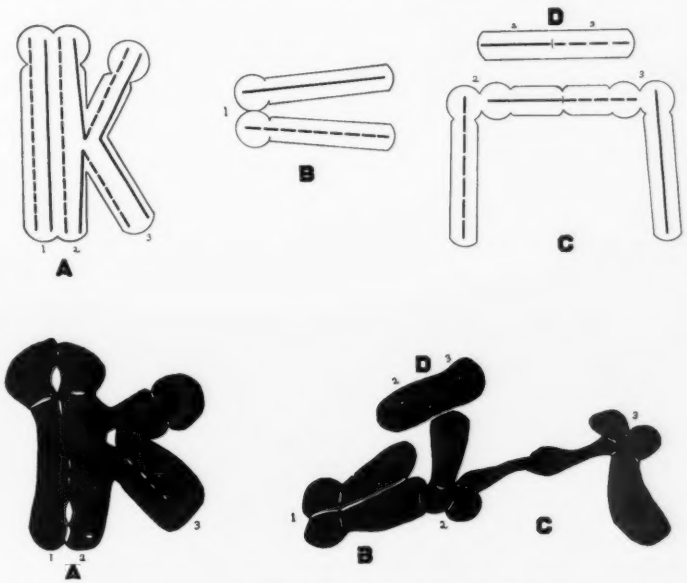

FIG. 31.—Diagrammatic interpretation of simple bridging in Inglescombe Yellow. *A* (solid black): enlargement of fig. 28. Diagrammatic interpretation of this configuration is labeled to correspond with the drawing. *B*, *C*, and *D* (solid black): enlarged from fig. 29*B* (chromosome groups 2*a* and 2*b*). Diagrams labeled to correspond with drawings. In the drawings and diagrams the homologues have been labeled 1, 2, and 3. Each of these becomes double in anaphase as shown in *B*, *C*, and *D*. Solid and broken lines have been used to denote individual chromatids. It is believed that metaphase trivalent configurations, like the one shown at *A*, give rise to anaphase complexes like the ones shown at *B*, *C*, and *D*. A number of cases of bridging have been analyzed in which all chromosomes and fragments were accounted for. Analyses in every case were consistent with the interpretation given in this figure.

TELOPHASE AND INTERPHASE I.—Particular attention was paid to the fate of chromosome fragments and other chromatin material not included in the daughter nuclei following the first and second divi-

sions. Such chromatin material is apparently never incorporated into any of the nuclei. By interphase I the excluded chromosomes, or parts of chromosomes, were altered in appearance and displayed strong affinity for stains. Sometimes laggards (in bridges or otherwise) were seemingly cut in half by the developing cell plate (fig. 29*D*). In 75 first interphase dyads the number of extra nuclear chromatin bodies per dyad varied from 0 to 6. The average number per dyad was 2.09, 12 per cent having no chromatin bodies and 4 per cent having six bodies per dyad. These chromatin bodies were the remains of excluded chromosomes or portions of chromosomes. In some cases large masses of extra nuclear chromatin had apparently been derived from whole bivalents or trivalents (fig. 29*C*). Counts of extra nuclear chromatin bodies were limited to those dyads in which situations like those shown in figure 29*C* and *D* did not occur. In other words, only completely separated bodies were counted.

METAPHASE II.—Second metaphases were characterized by the irregular form of the chromosomes, which were often similar to the first metaphase types. It was not possible to make accurate chromosome counts at this stage.

ANAPHASE II.—Bridging occurred frequently during the second anaphase, the chromatin strands often appearing as though under considerable tension. The extra nuclear chromatin bodies formed during the first meiotic division could be distinguished from the other chromosome elements during the stages of the second division by their form and staining reactions.

TELOPHASE AND INTERPHASE II.—After development of the microspore nuclei, the extra nuclear chromatin bodies generally assumed a somewhat spherical shape, staining heavily (fig. 30*B*). Unlike certain triploid tulips, Inglescombe Yellow forms microspore tetrads of quite normal appearance. Typical micronuclei were rarely observed. These were entirely distinct from the extra nuclear chromatin bodies described in this paper. The microspore nuclei of a single tetrad frequently differed in size, but the whole form of the tetrad was rather regular.

The distribution of the extra nuclear chromatin bodies in 2895 microspores from eight anthers of two bulbs of Inglescombe Yellow is given in table 3. The distributions obtained were remarkably uni-

form, even in random samples of only 100 microspores each. For the 2895 microspores recorded in table 3 the average number of extra nuclear chromatin bodies per microspore was approximately 0.8515, or 3.40 bodies per group of four microspores. In the compilation of this table both free microspores and those still organized in tetrads were included. Cytokinesis, however, had been completed in every

TABLE 3

DISTRIBUTION OF EXTRA NUCLEAR CHROMATIN BODIES IN 2895 MICROSPORES FROM EIGHT ANTHERS OF TWO BULBS OF INGLESCOMBE YELLOW. IN PLANT NO. 1 THE MICROSPORES WERE MOSTLY IN DEFINITE TETRADS, BUT IN NO. 2 SOME MICROSPORES WERE STARTING TO SEPARATE. AVERAGE NUMBER OF BODIES PER MICROSPORE WAS 0.8515

PERCENTAGE OF TOTAL MICROSPORES EXAMINED

No. bodies per spore	Plant no. 1, anthers 1 to 3			Plant no. 2, anthers 1 to 5					Average in percentage
	No. 1	No. 2	No. 3	No. 1	No. 2	No. 3	No. 4	No. 5	
0.....	40.2	39.5	40.5	36.0	40.2	39.1	39.6	42.5	39.7
1.....	38.4	39.0	41.5	42.3	36.9	41.3	41.0	40.5	40.1
2.....	15.4	18.5	14.0	16.0	17.6	14.5	15.6	14.0	15.7
3.....	4.6	2.5	2.0	4.6	4.5	3.8	3.6	2.5	3.5
4–5.....	1.2	0.5	2.0	1.0	0.6	0.3	0.0	0.0	0.7
Total no. examined	388	400	200	300	487	600	300	220	.....

case. In table 4 the frequency of extra nuclear chromatin bodies in 326 tetrads is given. These tetrads constituted 1304 of the microspores recorded in table 3. The average number of bodies per tetrad was approximately 3.45.

LATER STAGES.—Only limited studies have been made in material collected at a later stage than that immediately following separation of the microspores. In bulbs dug about nine weeks after those recorded in table 3 it was found that the extra nuclear chromatin bodies still persisted. They were best demonstrated in material fixed in Carnoy's fluid and stained in hot aceto-carmine. The bodies were smaller on the average and more homogeneous in structure at these late stages. The data in table 5, while very limited, show that differ-

ent triploids do not have the same frequencies of extra nuclear chromatin bodies in their microspores. It was possible to separate

TABLE 4

FREQUENCIES OF EXTRA NUCLEAR CHROMATIN BODIES IN 326 TETRADS OF MICROSPORES OF INGLESCOMBE YELLOW. AVERAGE NUMBER PER TETRAD WAS 3.45

No. of extra nuclear chromatin bodies per tetrad	No. of tetrads observed	Percentage of total tetrads
0	15	4.6
1	34	10.4
2	52	15.9
3	84	25.7
4	62	19.0
5	36	11.0
6	18	5.5
7	12	3.6
8	6	1.8
9	4	1.2
10 to 12	3	0.9

TABLE 5

DISTRIBUTION OF EXTRA NUCLEAR CHROMATIN BODIES IN MICROSPORES OF THREE TRIPLOID (3N EQUAL 36) TULIPS. MICROSPORES IN LATER STAGE OF DEVELOPMENT THAN THOSE RECORDED IN TABLES 3 AND 4

No. bodies per spore	Percentage of total microspores		
	Inglescombe Yellow	Inglescombe Pink	Cardinal Manning
0	47.0	19.3	84.6
1	44.0	32.9	12.6
2	7.0	24.1	1.3
3	1.0	16.4	0.0
4	1.0	5.4	0.0
5	0.0	0.9	0.0
6	0.0	0.6	0.0
Total no. examined	100	310	150

these varieties from one another on this basis alone, even when detailed counts were not made.

Discussion

The diakinetic and metaphase I chromosome configurations observed in Inglescombe Yellow are of the same general type observed by NEWTON and DARLINGTON (4) in triploid *Tulipa*. These workers found from three to ten trivalents at metaphase in each of the varieties worked with. In Inglescombe Yellow twelve trivalents per pollen mother cell were not rare. This tulip is therefore probably essentially autotriploid.

The trivalent associations occurring in Inglescombe Yellow are generally of the type to be expected on the basis of NEWTON and DARLINGTON'S observation that pairing of three homologous chromatids never occurred between all three threads at any one point. Configurations like those shown in figures 27 and 28, however, are hard to explain except on some such basis as that presented in figure 31. It seems very likely that breaks do occasionally occur in chromosome bridges at anaphase I in such a way that kinetochore-bearing fragments are incorporated in the daughter nuclei. In support of this view is the occurrence of a fragment chromosome in a seedling derived from a cross in which Inglescombe Yellow served as the pollen parent.

NEWTON and DARLINGTON (4) observed in polyploid tulips and hyacinths that during the diakinetic contraction small bodies of chromatin began to appear in the nucleus which were still visible at anaphase. These bodies were regarded as chromosome fragments. The origin of such fragments was supposed to take place between diplotene and diakinesis as the result of an unusual strain upon a single chiasma. In Inglescombe Yellow fragments have not been observed until the beginning of anaphase I.

NEWTON and DARLINGTON did not refer to the phenomenon of bridging and fragmentation as being related. Recently, however, DARLINGTON (1) has referred to some unpublished work of UPCOTT'S on bridging and fragmentation in diploid and triploid *Tulipa*. Some of the figures illustrate conditions very similar to those described in the present paper. SMITH (5) has described a process of chromosome fragmentation and bridging in *Trillium erectum* L. that seems to be very similar to behavior observed in the present study. A crossover between a normal chromosome and one with an inverted segment in the long arm was apparently the cause of the condition in *Trillium*.

In Inglescombe Yellow translocations between homologous chromosomes in trivalent association are apparently responsible for the development of bridges and fragments in anaphase I.

There can be no doubt that the extra nuclear chromatin bodies observed in microspores of Inglescombe Yellow follow a regular distribution (table 3). These bodies are derived from chromosomes and chromosome fragments excluded from daughter nuclei in the first and second meiotic divisions. From a consideration of the number of ways in which these extra nuclear chromatin bodies can originate or be increased in number, it is obvious that a mathematical interpretation of the data in tables 3 and 4 would be difficult. It is significant, however, that the numerical distribution of these bodies in the microspores is highly characteristic. They furnish an index as to the amount of certain types of irregular behavior occurring in the different triploids.

Summary

1. The cottage tulip, Inglescombe Yellow, behaves cytologically as an autotriploid, twelve trivalents at metaphase I not being rare.

2. In anaphase I chromosome bridges and fragments sometimes occurred. These apparently resulted from translocations in the previous prophase between homologous chromosomes involved in trivalent configurations. The bridges consisted of kinetochore-bearing portions of two homologous daughter chromosomes distally united. The released fragments were without kinetochores and consisted of united portions of the same daughter chromosomes involved in the bridges. Kinetochore-bearing fragments were sometimes formed.

3. Lagging univalents, fragments, portions or all of bridge configurations, etc. were often excluded from the daughter nuclei in interphase I. These formed darkly staining extra nuclear bodies in interphase. In anaphase II further bridging and fragmentation occurred, resulting in the formation of additional extra nuclear bodies. Occasionally during cytokinesis, following both the second and first divisions, some of the extra nuclear masses of chromatin were apparently cut in two by the developing cell plates.

4. The microspores were generally organized in tetrads. The frequency distributions of extra nuclear chromatin bodies in the microspores were characteristic. Approximately 40 per cent of the micro-

spores contained no extra bodies, 40 per cent contained one body per spore, 16 per cent contained two per spore, and the remaining 4 per cent from three to five per spore.

5. It was possible to separate three different triploid tulip varieties on the basis of the frequency distributions of extra nuclear chromatin bodies in their microspores.

The writer acknowledges his indebtedness to Dr. RONALD BAMFORD for valuable suggestions, criticisms, and unfailing interest during the progress of this work.

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ASCORBIC ACID IN THE AVENA COLEOPTILE

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It has been shown (**3, 5, 6**) that vitamin C (ascorbic acid) may act as a growth factor in plants. It became of interest to investigate the distribution of this substance in the *Avena* (oats, variety Victory) coleoptile, since this organ has been more extensively studied from the standpoint of growth phenomena than any other plant organ. It was desired to determine the ascorbic acid distribution in the coleoptile and to see whether the content changes when certain external conditions are altered. It was proposed also to investigate the possible relations between the growth effects of auxin, one of the plant growth hormones, and ascorbic acid.

The plant tissues were cut from the seedlings in the darkroom and quickly crushed in 3 per cent metaphosphoric acid, the macerate filtered, the filtrate acidified with 1 cc. 0.01 N HCl, and the ascorbic acid content determined by titration with 2-6 dichlorophenolindophenol (20 mg. per cent) from a microburette. The dye solutions were previously standardized with known amounts of pure ascorbic acid (Roche). After titrating for the reduced ascorbic acid, the solutions were treated with H_2S to reduce all oxidized ascorbic acid present, and the titrations repeated. The difference between the two titrations gave the amount of oxidized ascorbic acid originally present in the filtrate.

Coleoptiles were removed from 72-hour old etiolated seedlings, the primary leaves removed, and the coleoptiles cut into three sections, apical, middle, and basal portions, each section approximately 10 mm. in length. Their reduced and oxidized ascorbic acid contents were determined as outlined, both on the basis of dry and of wet weight. On the average, one hundred coleoptiles per determination were used. The wet weight determinations are shown in table 1.

It is evident that there is a distinct gradient of concentration of reduced ascorbic acid in the *Avena* coleoptile. It is indicated that there is also a gradient of oxidized, which is opposite that of the

reduced, but this latter point demands more determinations, due to the one exception, namely experiment 3. The dry weight determinations are shown in table 2.

TABLE 1

DISTRIBUTION OF REDUCED AND OXIDIZED ASCORBIC ACID IN AVENA COLEOPTILES (MG. PER GM. WET WEIGHT)

Experiment	Reduced ascorbic			Oxidized ascorbic		
	Tips	Middles	Bases	Tips	Middles	Bases
1	0.415	0.343	0.294	None	0.076	0.087
2	0.592	0.428	0.303	None	0.028	0.061
3	0.614	0.414	0.313	0.056	0.053	0.047
4	0.500	0.448	0.403	None	None	None
5	0.519	0.434	0.350	.....	.....	.....
6	0.698	0.555	0.455	.....	.....	.....
Average	0.556	0.437	0.353	0.014	0.039	0.049

TABLE 2

DISTRIBUTION OF REDUCED AND OXIDIZED ASCORBIC ACID IN AVENA COLEOPTILES (MG. PER GM. DRY WEIGHT)

Experiment	Reduced ascorbic			Oxidized ascorbic		
	Tips	Middles	Bases	Tips	Middles	Bases
1	21.80	17.90	16.95	None	3.00	4.85
2	19.91	18.95	13.97	None	1.25	2.63
3	17.90	20.50	18.65	.....	.....	.....
4	19.95	19.84	17.52	2.17	2.43	2.32
5	25.50	24.30	22.60	None	None	None
Average	21.01	20.29	17.34	0.54	1.67	2.45

The distribution of ascorbic acid in the first internodes of *Zea* seedlings (VAN OVERBEEK, unpublished) is similar to that in *Avena* coleoptiles.

BONNER (2) has presented data showing the distribution of dry weights and protein in the *Avena* coleoptiles. These data are shown in table 3.

From table 3 it is seen that basal sections contain more dry weight per unit length than apical sections. Since BONNER's data show a practical equality of percentage protein distribution per unit dry weight, the ascorbic acid distribution per unit weight of cytoplasm would follow that of dry weight as shown in table 2. From the difference between wet and dry weights, the ascorbic acid distribution per unit weight of cell sap is seen to be identical with that of wet weight determinations.

It has been claimed that ascorbic acid is found in higher concentration in those parts of plants which contain higher amounts of

TABLE 3

DISTRIBUTION OF DRY WEIGHTS AND PROTEIN IN AVENA COLEOPTILE (FROM BONNER)

	TIP 5 MM.	NEXT 6 MM.	NEXT 6 MM.	BASE 13 MM.
	Mg. protein per mm.			
Average of 4 determinations.	1.10×10^{-2}	1.21×10^{-2}	1.36×10^{-2}	1.46×10^{-2}
	Mg. dry weight per mm.			
Average of 3 determinations.	3.9×10^{-2}	4.9×10^{-2}	5.3×10^{-2}	5.5×10^{-2}
Mean percentage protein...	28	25	25.5	27

chlorophyll (**4, 8**). The synthesis of ascorbic acid in plants, however, is not dependent upon the presence of chlorophyll and photosynthesis, as will be shown later, but may be formed if photosynthesis occurs. It probably could be formed from hexose added to the medium in the absence of chlorophyll.

The following experiments prove that ascorbic acid may be formed in the absence of chlorophyll. Etiolated *Avena* coleoptiles (1000 plants) from seedlings grown in the darkroom with occasional illumination with orange-red light of wave length no shorter than 575 mμ were extracted several times with hot methanol, the extracts filtered through sintered glass, and the chlorophyll transferred to

petroleum ether. The ether extract was passed through a small Tswett sugar column. A faint green band in the upper part of the chromatogram indicated the presence of chlorophyll. Methanol extracts of apical sections showed a visibly greater amount of chlorophyll than basal sections, showing a distribution gradient of chlorophyll. This fact was borne out by spectrophotometric determinations. Apical and basal sections of 500 coleoptiles grown in (1) the orange-red illumination of the darkroom, and (2) complete darkness, were extracted five times with hot methanol, the extracts filtered through sintered glass, and made up to equal volumes (3 cc.) with methanol. The 3 cc. samples were transferred to a standardized cuvette and the extinction coefficients measured in a spectrophotometer, using a Neon arc as light source, and choosing a line at 6599 A. The extinction coefficient of a known concentration of pure chlorophyll being known (0.476 for 10 mg. per liter of methanol in the above cuvette), the amounts of chlorophyll could be determined. The tips of coleoptiles grown in the orange-red light contained 0.86 gamma (0.00086 mg.) per gm. of wet weight, while the basal sections contained no detectable chlorophyll (the limits of sensitivity of the instrument being about 0.1 gamma for the 3 cc. samples used). The coleoptiles grown in complete darkness contained no measurable amounts of chlorophyll, although the leaves contained some (4 gamma per gm. as compared with 9 gamma in leaves grown in orange-red light).

Coleoptiles grown in complete darkness were found to contain ascorbic acid, however, the distribution gradient from tip to base existing but the absolute amounts being smaller than in plants grown in the orange-red light or in the greenhouse (see later). Apical sections of coleoptiles from seedlings grown in the complete dark contained 0.165 mg. reduced ascorbic per gm. wet weight, while basal sections contained 0.147. This is about 30 per cent (for tips) of the amounts found in coleoptiles from seedlings grown in orange-red light, and about 20 per cent of the amounts found in coleoptiles from seedlings from the greenhouse (see later). Hence photosynthesis is not essential for the formation of ascorbic acid in plants, but when chlorophyll is present, it is distributed in the same way as ascorbic acid, that is, it is in higher concentration in apical parts

of the coleoptile, as shown by the spectrophotometric determinations.

The seeds of germinating seedlings of *Avena* were separated from the roots and the shoot, and analyzed for ascorbic acid. None was present. The coleoptiles of the same seedlings contained 0.54 mg. per gm. wet weight.

It was just stated that light increases the ascorbic acid content of coleoptiles. This is shown by the data of table 4.

The coleoptiles of the plants exposed to light were very short and bright green in appearance, while those from the darkroom were

TABLE 4

EFFECT OF LIGHT (GREENHOUSE) AND AGE ON ASCORBIC CONTENT OF AVENA COLEOPTILES

AGE (HOURS AFTER SOAKING SEEDS)	IN LIGHT 36 HOURS	IN DARKROOM
72	0.750 mg./gm.	0.460 mg./gm.
96	0.430	0.354
172	0.327	0.314

only a faint yellowish green. It can be seen from these experiments that when plants contain chlorophyll and are exposed to light, ascorbic acid is formed in the coleoptile, accumulating in the tip more than in other parts. This may mean that the seat of synthesis is at the tip, as in the case of auxin. The precursor is probably mobilized from the seed reserves. That age decreases the ascorbic acid content is understandable on the basis that the seed reserves are exhausted during growth, and the precursor is depleted. Assimilation may replace this depletion, as seen by the preceding data (table 4).

The explanation for the decrease of the reduced and the increase of the oxidized ascorbic acid from tip to base of the coleoptile suggested a gradient of oxidation from tip to base, catalyzed possibly by an enzyme in the plant. This possibility was examined. The following protocol shows the result of one such experiment.

PROTOCOL.—Coleoptile sections from 100 seedlings were cut into tip, middle, and base sections, and weighed. They were macerated

in 3 per cent metaphosphoric acid, filtered, and the filtrates diluted with M/15 PO_4 buffer (at pH 6.0; 0.01 M cystine added to stabilize the ascorbic) in such a way that the inequality in weight of the sections was balanced by appropriate dilution. Ascorbic acid (Roche) was added to each of the three lots, and to the buffer control (1 cc. of a solution made by dissolving 0.0114 gm. ascorbic in 10 cc.). The time was recorded. One cc. of each lot was diluted with 5 cc. of 3 per cent metaphosphoric acid, and titrated at intervals with 0.001 M iodine solution. The results in table 5 are tabulated in cubic centimeters of iodine, and represent the relative amounts of ascorbic acid remaining in each solution.

TABLE 5

GRADIENT OF ENZYMATIC DESTRUCTION OF ASCORBIC ACID IN AVENA COLEOPTILES (CC. IODINE)

TIME (MINUTES)	BUFFER	TIPS	MIDDLES	BASES
40	1.80	1.80	1.66	1.34
60	.....	1.75	1.61	1.26
135	1.66	1.57	1.45	0.94
255	.....	1.36	1.30	0.64

A similar experiment showed essentially the same result, as did a third. In the latter experiment, however, the buffer solution destroyed more ascorbic acid than did the plant tissue. Three experiments were run in which the pH was 6.6. No difference in destruction was found between apex and base. This may be explained by the fact that spontaneous oxidation of ascorbic acid occurs more readily at higher pH values, and could thus obscure small differences in destruction in the coleoptile. It is demonstrated by these experiments that the tips of coleoptiles oxidize less ascorbic acid than the bases, when the pH is adjusted to approximate that of the plant sap.

Since it is indicated in these experiments that the destruction of ascorbic acid is a function of the pH, it was thought possible that the gradient of destruction might be explained by a corresponding pH gradient in the plant. The evidence does not favor this concept, as BABIČKA (1) reported that exudates from apical cut sur-

faces of stems of several different plants are more acid than those from basal cut surfaces. BONNER moreover reported no such pH gradient in the *Avena* coleoptile. It is interesting that the pH of the plant sap (about 6.0) is just low enough to prevent rapid destruction of ascorbic acid. Experiments have shown that the ascorbic acid is not destroyed very rapidly in coleoptile extracts until the pH is higher than 6.0.

VAN OVERBEEK (**7**) has shown that heteroauxin is destroyed more by basal parts of coleoptiles than by apical parts, and that basal parts of mesocotyls contain more oxidative enzymes (peroxidase) than apical parts. Whether the gradient of oxidative enzyme for ascorbic destruction in the coleoptile bears any relation to such a peroxidase distribution remains to be shown.

The relation of ascorbic acid to plant growth is not yet known. It is known that ascorbic acid increases regeneration of willow branches, increases germination, and accelerates growth when supplied to the root system (**3, 5, 6**). Hence it was thought interesting to investigate the possible role of ascorbic acid as a growth hormone, or whether it acts together with a growth hormone such as auxin. The activity of ascorbic acid as a growth hormone was therefore tested, using the standard *Avena* technique (**9**). Ascorbic acid in agar blocks buffered at pH 5.0 to 6.0 had no effect on the curvatures, although it acted as an acid when unbuffered. In this case curvatures were produced, but since any other acid will presumably do this because of effects on the dissociation of regenerated auxin in the plant, it was concluded that ascorbic acid is not a cell elongation hormone like auxin. Ascorbic acid mixed with indole-3-acetic acid was likewise inactive in the *Avena* test as ordinarily performed. There is some possibility that it can act as a "food factor" in growth (**9**), since small curvatures were produced when auxin was made no longer limiting in its effect on growth. (This can be done by supplying high concentrations to the decapitated coleoptile for some time before applying the agar blocks containing the auxin to be tested.) Ascorbic acid in buffered solutions, stabilized with cystine, and with or without auxin, had no effect on the pea test for growth hormones (**9**). It was concluded that ascorbic acid must act in some other way on growth in plants than on the auxin mechanism concerned

in cell elongation. It is possible that the growth effects claimed for ascorbic acid are related to cell proliferation, as BONNER (unpublished) has found effects of ascorbic acid on the growth of pea embryos and buds in nutrient solutions.

Summary

1. Ascorbic acid is present in considerable concentrations in *Avena* coleoptiles from etiolated seedlings.

2. On the basis of wet weight determinations, reduced ascorbic acid is present in higher concentrations in tips than in bases of coleoptiles, and oxidized ascorbic acid shows the reverse gradient of distribution.

3. The distribution of ascorbic acid in the coleoptile corresponds with the distribution of chlorophyll in plants grown in the light, but does not depend upon chlorophyll either for its presence or for its distribution in the coleoptiles of plants grown in the dark.

4. Light increases the concentration of ascorbic acid in the coleoptile, and aging decreases it.

5. Ascorbic acid is not present in the germinating seed, but is synthesized in the coleoptile from a precursor in the seed.

6. Reduced ascorbic acid is oxidized more by extracts from basal sections of coleoptiles than by extracts from apical sections. This destruction gradient corresponds with the destruction gradient of auxin in the coleoptile.

7. Ascorbic acid is not a cell elongation hormone like auxin, nor does it facilitate the action of auxin in the standard *Avena* or pea tests.

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POLARIZED GROWTH AND CELL STUDIES IN THE FIRST INTERNODE AND COLEOPTILE OF AVENA IN RELATION TO LIGHT AND DARKNESS

GEORGE S. AVERY, JR., PAUL R. BURKHOLDER, AND HARRIET B. CREIGHTON

(WITH EIGHT FIGURES)

Introduction

Much of the influence of light upon growth and development in plants has recently been explained by the role of growth hormones. Since rate of growth in *Avena* has been shown to be proportional to the concentration of growth hormone (**21**), within certain limits, differential distribution or inactivation of these growth regulating substances might be expected to modify the relative growth and development of the component parts of the seedling. Determinations of growth hormone in coleoptile tips of *Avena* seedlings have shown that the values obtained for irradiated plants are much lower than for plants maintained in darkness (**7, 8, 17**). Many differences in the external appearance of plants grown in light and darkness are well known and have been described at length in the literature. The question naturally arises as to the proportionate influence of light upon the cellular activities in growth; that is, cell multiplication and cell enlargement.

The nature of the first internode of the *Avena* seedling and the vascular relationship of its several parts have been described (**3**), and the gross form and microscopic structure of the coleoptile (grown in a darkroom under conditions favorable for its use as a standard test object for the quantitative determination of plant hormones) is now well known (**1**). Cell elongation and increase in cell number take place only in the plane of the long axis; that is, growth is polarized. The cell and tissue responses in the growth of the coleoptile exposed to light have not yet been investigated. Studies of phototropism of the coleoptile and growth of the first internode of *Avena* in relation to light have indicated marked differences in sensitivity and growth

response of the two organs. The coleoptile is very sensitive to the blue region of the spectrum, but not to the red (14); its growth in weak red light is about the same as in darkness. The first internode, however, attains considerable length when grown in complete absence of light, but its growth is appreciably shortened by exposure to light of all wave lengths. The influence of different dosages of light, as to quality and amount, upon the proportionate development of the coleoptile and internode, as well as the number and size of the cells in these organs, is unknown.

The purpose of this study was to investigate: (*a*) the influence of light and darkness upon growth of the coleoptile and first internode of different varieties of oats; (*b*) the influence of different intensities of light upon the first internode; (*c*) the influence of different intensities of light upon the coleoptile; and (*d*) the influence of light and darkness upon cell division and cell enlargement in the coleoptile and first internode throughout their ontogeny.

Investigation

Length of first internode and coleoptile of different varieties of oats

Size of the first internode and coleoptile of several varieties of oats was determined for seedlings grown (1) in complete darkness and (2) for a short time in light and then in darkness. The seeds, obtained through the courtesy of Mr. T. R. Stanton of the United States Department of Agriculture, Washington, D. C., were planted in moist clear quartz sand contained in glass vessels. One series was placed immediately in a darkroom and kept there until the first foliage leaves burst through the coleoptiles. The other series was subjected to daylight in a greenhouse during the first ten hours after planting and then placed in the darkroom. The darkroom temperature was 80° F. and the relative humidity 88–90 per cent. Measurements of the length of first internodes and length and diameters of coleoptiles are recorded in table 1.

The first internode failed to elongate to any extent in the series receiving a preliminary light exposure; in this same series also the embryonic foliage leaves burst through the coleoptiles about two days earlier than those of plants grown in complete darkness, the

latter group requiring about eight days. Decided differences in length of first internode and coleoptile were shown by the different varieties. Grown in complete darkness, most varieties possessed coleoptiles which were longer than the first internodes; a few varieties, such as Victory, Albion, and Hairy Culberson, gave the reverse relationship. All varieties exhibited great elongation of the first inter-

TABLE 1

SIZE OF FIRST INTERNODE AND COLEOPTILE IN DIFFERENT VARIETIES OF OATS SEEDLINGS GROWN IN DARKNESS WITH AND WITHOUT PRELIMINARY EXPOSURE TO DAYLIGHT; TEMPERATURE 80° F., RELATIVE HUMIDITY 90

Variety	Complete darkness				Light exposed		
	No. plants measured	First internode length	Coleoptile length	Coleoptile diameter	No. plants measured	Coleoptile length	Coleoptile diameter
Black Tartar	20	30.7	53.8	1.5	23	38.2	1.6
Storm King	6	57.0	64.4	1.8	21	52.5	1.7
Black Mesdag	19	18.6	47.8	1.7	23	34.7	1.7
Victory	13	47.2	44.2	1.5	20	40.7	1.5
Navarro	23	22.4	55.6	1.7	22	44.4	1.6
Carleton	22	46.0	66.7	1.5	22	49.8	1.6
Victoria	23	42.8	62.7	1.6	22	47.5	1.4
Fulghum	16	23.8	49.8	1.7	23	52.8	1.6
Richland	15	41.6	50.2	1.5	24	43.5	1.5
Markton	21	46.9	59.1	1.6	21	45.7	1.5
Coastblack	21	40.0	55.6	1.6	22	55.5	1.6
Bond	23	23.7	56.5	1.6	24	45.0	1.6
White Tartar	22	45.5	54.3	1.6	24	46.8	1.5
Albion	18	51.3	48.6	1.5	24	42.7	1.5
Support	23	35.0	64.2	1.6	15	48.4	1.6
Golden Giant	22	12.0	46.2	1.5	21	44.3	1.4
Hairy Culberson	20	55.0	50.2	1.5	23	42.0	1.5
Aurora	22	23.7	50.8	1.5	15	34.3	1.5
Average	.....	36.8	54.4	1.55	.....	44.9	1.58

node in complete darkness, while exposure to bright light at the beginning of germination inhibited growth in this region almost completely. The average total length of the seedling shoot (first internode plus coleoptile) at the time the foliage leaves emerge is, in the completely darkened plants, more than twice as great as in those given preliminary illumination. The significance of this fact from the standpoint of survival of seeds germinating in darkness at relatively great depths in the soil is apparent. Those plants able to reach

light before exhaustion of the stored food supply would appear to have a decided advantage.

Light intensity and length of first internode in seedlings of Victory oats

In order to determine what relationship might exist between length of the first internode and intensity of the light under which they germinate, Victory oat seeds were placed upon wet coarse cheesecloth tied over glass vessels filled with distilled water (fig. 1).

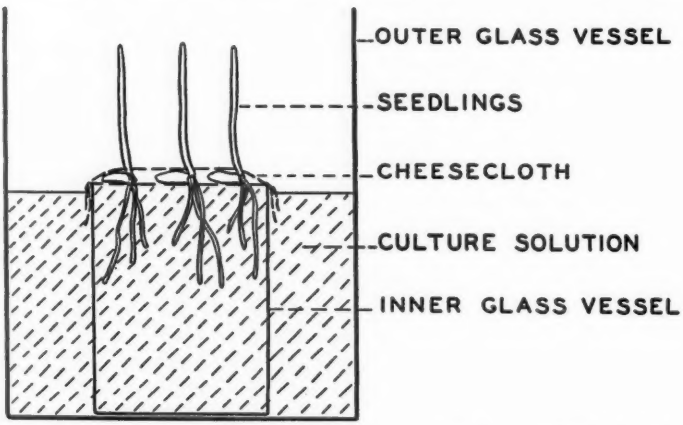

Fig. 1.—Culture vessels for growing seedlings: a small glass vessel filled with culture solution was placed inside a larger vessel containing the same solution and the level of the water in germinating vessel was maintained so that the seeds were kept well moistened. Seeds planted between two layers of coarse cheesecloth (held in place with a rubber band) grow well without further attention.

With this simple apparatus it was possible to place dry seeds upon the wet coarse cheesecloth surface and leave the seedlings to grow without further attention under constant conditions in a room maintained at 80° F. and 90 per cent relative humidity.

The first experiment was performed with a single 5 watt Neon glow lamp operated on a 110 a.c. line. The lamp was placed at one end of the darkroom and the seedlings germinated at different distances from the lamp. Further reduction in intensity was obtained by using neutral filters for the lowest two intensities. The light beam

was reflected vertically downward on to the seedlings by means of plane glass mirrors at an angle of 45°. Average measurements of first internodes of plants grown under different intensities and in darkness are given in table 2 and figure 2.

In table 2A the relative intensity is given when the highest value is set at 1; the maximum energy incident upon the plants was not accurately determined, but was approximately 0.1 erg/mm.²/sec. Pronounced shortening of the first internodes was brought about by

TABLE 2

FINAL LENGTH OF FIRST INTERNODE IN VICTORY OATS GROWN UNDER DIFFERENT INTENSITIES OF LIGHT; TEMPERATURE 80° F, RELATIVE HUMIDITY 90

A. LIGHT FROM 5 WATT NEON GLOW LAMP

Relative intensity.....	0	0.01	0.02	0.059	0.174	1.0	
Number of plants.....	19	26	25	23	23	23	
Length of internode (mm.)	67.1	26.4	17.4	16.8	12.2	4.8	

B. LIGHT FROM 7.5 WATT MAZDA LAMP

Intensity (ergs/mm.²/sec.)	0	0.027	0.154	0.224	0.585	2.006	3.363
Number of plants.....	80	95	95	97	91	51	69
Length of internode (mm.)	71.2	40.4	24.6	18.7	12.7	8.34	2.20

comparatively small amounts of radiant energy in this region (red) of the spectrum.

A similar experiment was performed with a 7.5 watt Mazda lamp as the source of radiation. The energy was measured with an Eppley thermopile and a Leeds and Northrop type HS galvanometer. The energies and corresponding lengths of first internodes are given in table 2 and figure 3. An average first internode length of 71.2 mm. was attained in complete darkness. Marked shortening of the internode occurred under the lowest intensities used. The plotted data form a curve which shows pronounced effects of light at relatively low energy levels; comparatively little further shortening of the internode could be brought about by energy values greater than those plotted.

CELL NUMBER AND LENGTH OF COLEOPTILE IN VICTORY OATS IN RELATION TO INTENSITY OF LIGHT

Preliminary experiments were performed in the greenhouse to test the influence of daylight and darkness upon the final length attained

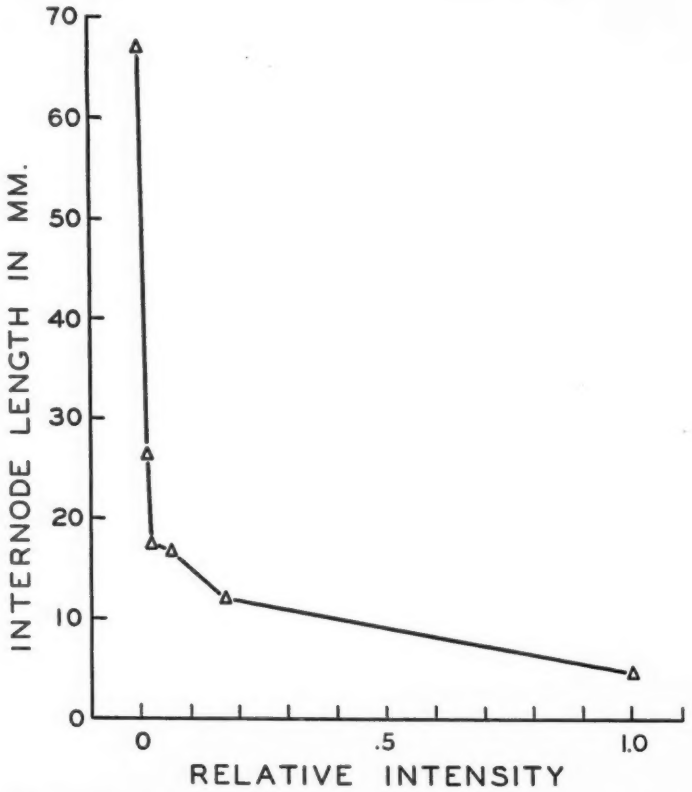

FIG. 2.—Length of first internode of *Avena* seedlings in relation to intensity of Neon light. An intensity of about 0.1 erg/mm.^{2}/sec. was sufficient to shorten the internodes to one-fourteenth the length attained in darkness (see table 2).

by *Avena* coleoptiles. Seeds were planted in water culture vessels inclosed under bell jars. One set was placed in darkness and the other allowed to grow in moderately bright light. After about one week,

the coleoptiles were measured. The average final length of twenty-five illuminated coleoptiles was 18.7 mm. Another set of six plants grown under six 300 watt Mazda lamps (screened through a 5 cm. water cell) reached 24.7 mm. in length. Controls in darkness attained a length of 37.4 mm.

The final length attained by coleoptiles under different intensities of light was studied by growing seedlings in water cultures exposed

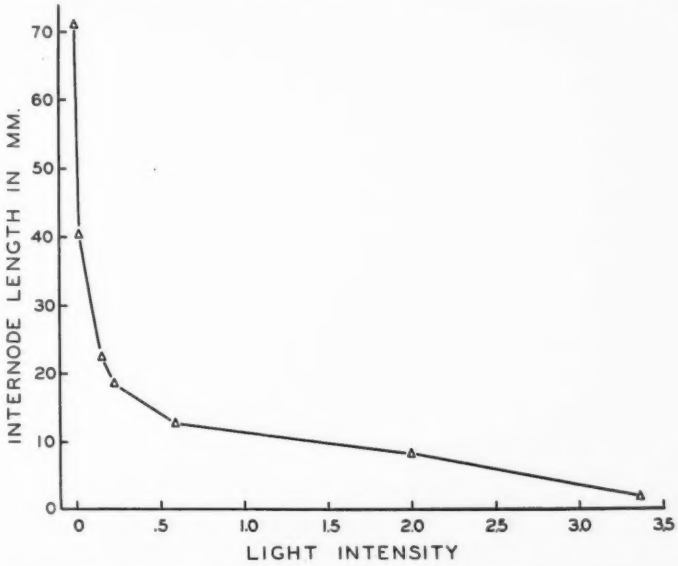

FIG. 3.—Final length attained by first internode of *Avena* in relation to intensity of Mazda light (see table 2). Intensities plotted as ergs/mm.²/sec.

to the light of a 6 inch mercury in quartz arc operated at 220 volts d.c. and 7.5 amperes. A plate glass water cell 5 cm. thick was placed between the arc and the plants for the purpose of filtering out the lethal ultraviolet and the infra-red radiation. Plants were grown in large metal boxes under seven different intensities obtained by varying the distance from the arc and by using black cloth screens. One set of plants was grown in darkness. The temperature about the plants was maintained at 80° F. in an air conditioned room.

After about four days the plants exposed to appreciable amounts of radiation had embryonic foliage leaves bursting through their coleoptiles, but those in the lowest intensities and in darkness required six or seven days for completion of coleoptile growth. All coleoptiles and internodes were measured for length, and then fixed in Craf solution (**19**) for cell studies.

The results of this study are presented in table 3. Marked shortening of the internodes was apparent under all intensities of light, but the coleoptiles were appreciably shortened only under

TABLE 3

FINAL LENGTH, AND CELL COUNTS IN COLEOPTILE (AND LENGTH OF FIRST INTERNODE) OF VICTORY OATS GROWN IN WATER CULTURE UNDER DIFFERENT INTENSITIES OF LIGHT FROM MERCURY IN QUARTZ ARC

Intensity (ergs/mm.²/sec.)	0.07	0.14	0.42	1.24	2.24	83.63
Length of coleoptile (mm.)	50.4	49.4	49.9	45.1	40.1	23.7
Number of cells* (each figure the average of 4–8 coleoptiles)	141	.....	150	.....	154	118
Length of first internode (mm.)	13.0	3.3	1.3	0.3	0	0
Number of plants	15	18	17	18	18	32

* Unelongated cells at tip of coleoptile not included in these cell counts.

the highest intensity used. Note the greater inhibition of internode elongation by radiation from a mercury arc as compared with the same intensity of light from a Mazda lamp (tables 2 and 3).

CELL NUMBER AND SIZE IN FIRST INTERNODE OF VICTORY OATS IN RELATION TO INTENSITY OF LIGHT

A selected number of typical first internodes, obtained in the experiment dealing with the influence of low intensities of Mazda light on polarized growth in this region of the oat plant, were imbedded in paraffin, sectioned at 14 μ, and stained with crystal violet. Counts were made of the number of cells in the fourth layer of cortical parenchyma from the level of divergence of the epiblast to that of the coleoptile (figs. 4–6). The averaged measurements are recorded in table 4 and figure 7. It may readily be seen (table 4) that the number of cells varies inversely with the intensity of light. Calculated values for the length of the cells show a decrease with increasing light intensity. In the graph (fig. 7) the curves for internode length, cell number,

and cell size run more or less parallel throughout the entire intensity range. In the plants grown in complete darkness, the cell number increases slightly more rapidly than the internode length, and the average increase in cell size is not so great as the increase in internode length. Since internode length equals cell number times cell size, it seems to follow that in darkness multiplication of cells is favored and

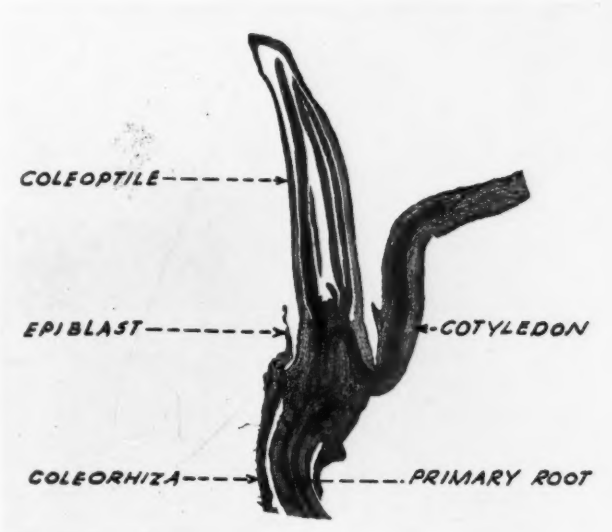

FIG. 4.—Median longisection of *Avena* seedling, grown in light for 36 hours, endosperm, etc., removed (see table 5).

the length of the cells does not increase beyond a certain maximum. The question arises whether the maximum size attainable by cells before division is initiated may be controlled by light. Further evidence along this line is presented in the following.

CELL DIVISION AND ELONGATION DURING EARLY STAGES OF GERMINATION IN LIGHT AND DARKNESS

Seeds of Victory oats were planted in water culture vessels, one set being kept in a darkroom and another set under light supplied by a 1000 watt Mazda lamp and filtered through a 5 cm. thick water

cell. The temperature was controlled at 80° F. in each of the air conditioned rooms. At stated intervals (table 5) plants were pickled

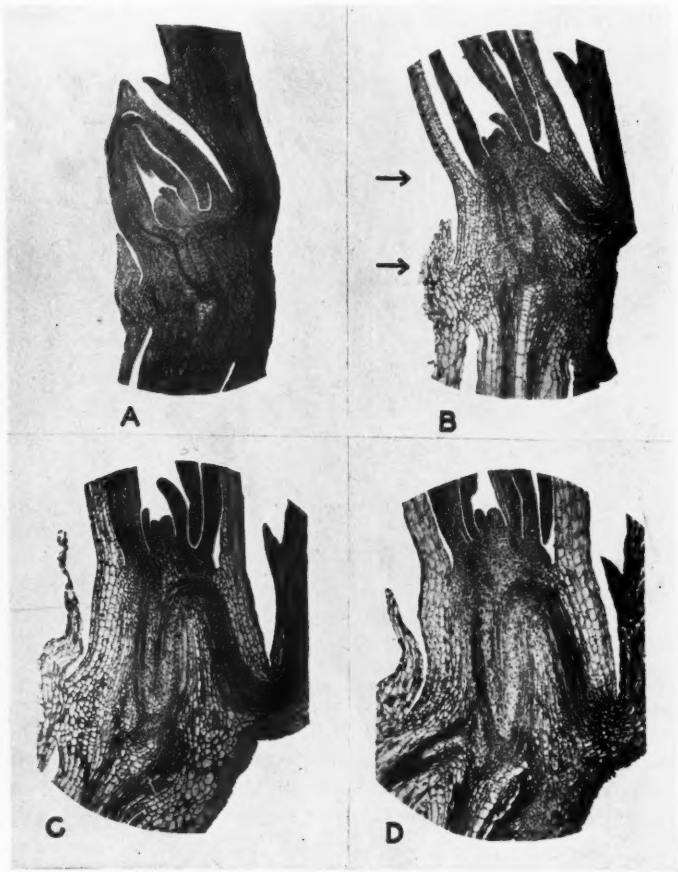

FIG. 5.—Median longisections of first internode, etc., of *Avena* seedlings, grown in light. Upper and lower limits of first internode indicated by arrows. *A* (embryo), after 6 hours; *B*, after 24 hours; *C*, after 36 hours; *D*, after 42 hours (see table 5).

in Craf solution and later imbedded in paraffin, sectioned, and stained with crystal violet. The lengths of the internodes and coleop-

tiles were measured on the slides, and the number of cells in the fourth row of the cortex in the first internode or median row in the coleoptile was counted. From these data the average size of cells was

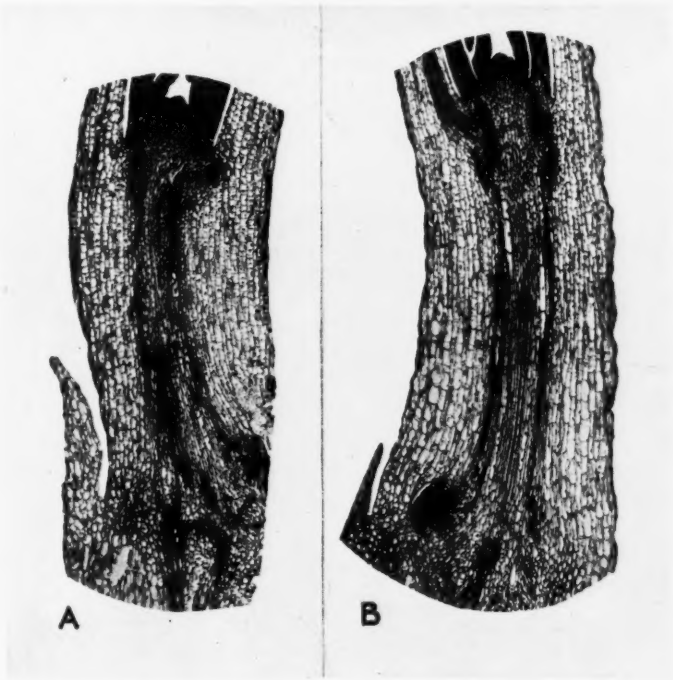

FIG. 6.—Longisections of first internode of *Avena* seedlings, grown in darkness. *A*, after 36 hours; *B*, after 42 hours (see table 5). In *B*, particularly, note the meristematic region (with smaller cells) in the upper portion of the internode. Whenever light conditions are such that the first internode elongates, it is at first meristematic throughout. As it grows longer, the meristematic region becomes localized in the upper end of the internode, in the zone just below the coleoptilar node.

computed. Five or six plants were used for determining each average figure presented in table 5 and figure 8. See also figures 4–6.

The data indicate a very small amount of polarized growth in the internodes of plants grown in light. Microscopic evidence indicates that no cell division and only moderate enlargement of the cells took

place. In contrast, the internodes kept in darkness elongated slightly during the first thirty-six hours after initial soaking in water, and thenceforth grew rapidly until a length of 46.4 mm. was attained

TABLE 4

FINAL CELL NUMBER AND SIZE IN FIRST INTERNODE OF VICTORY OATS GROWN IN WATER CULTURE UNDER DIFFERENT INTENSITIES OF MAZDA LIGHT

Intensity (ergs/mm.²/sec.)	Dark	0.154	0.585	2.006	3.363
Number of cells	159.0	57.7	47.3	34.5	25.0
Length of internode in mm. (on slides)	72.8	27.0	17.9	10.6	4.3
Computed length of cells (μ)	458	468	378	307	172
Number of plants	8	9	9	11	7

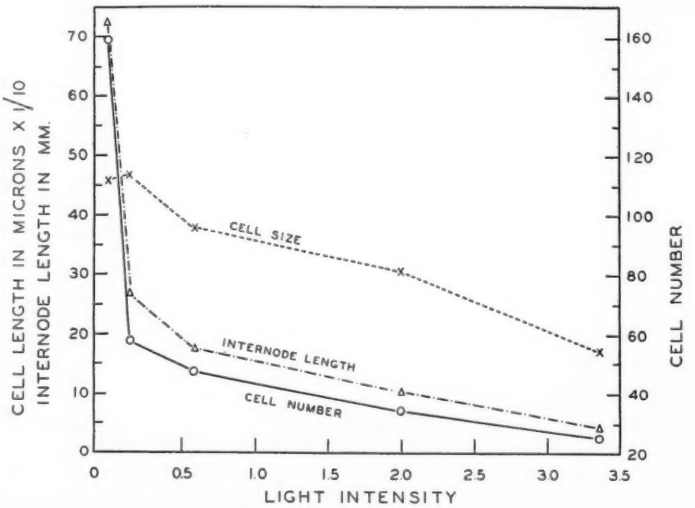

FIG. 7.—Cell number and cell size in first internode of *Avena* in relation to light intensity. Increased length of internode in progressively lower light intensities accounted for by increased numbers of cells as well as larger cell size; in complete darkness, however, the considerable further elongation of internode is due to larger number of cells resulting from further meristematic activity.

after eighty-six hours. Cell number increased a little faster than length of internode, hence cell length did not increase directly in proportion to length of internode in the dark-grown seedlings.

TABLE 5

LENGTH, CELL NUMBER, AND CELL SIZE OF FIRST INTERNODES AND COLEOPTILES OF AVENA SEEDLINGS AT DIFFERENT AGES IN LIGHT AND DARKNESS

	Age of seedling											
	6 hours		24 hours		36 hours		42 hours		86 hours		206 hours	
	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark
Internode cell number	19.5	18.6	19.8	20.5	19.0	31.5	22	42.2	19.8	144	.....	159
Internode length (mm.)	0.264	0.283	0.493	0.339	0.683	0.813	0.864	2.66	1.2	46.4	.....	72.8
Computed cell length (μ)	13.5	15.2	24.9	16.5	35.9	26.2	39.3	63.0	60.8	322	.....	458
Coleoptile cell no.	45	45	83	61	114	108	142	162	.....	.....	.....	.....
Coleoptile length (mm.)	0.831	0.787	1.836	1.228	3.654	3.657	7.45	5.746	.....	.....	.....	.....
Coleoptile, computed cell size (μ)	18.2	17.5	22.0	20.0	32.1	33.9	52.3	35.5	.....	.....	.....	.....

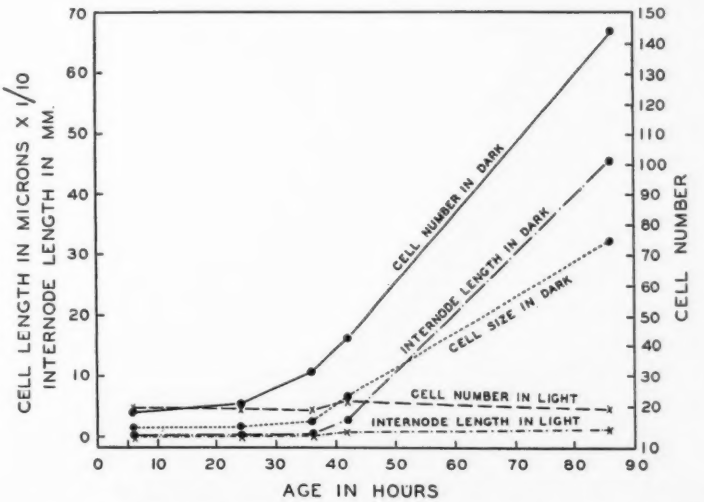

FIG. 8.—Length of first internode in *Avena* and number and size of its constituent cells at different stages during germination in light and darkness. Note that greater internode length in plants grown in darkness is due to increase in number as well as in size of cells. In contrast, internodes of plants grown in strong light (four feet from 1000 watt Mazda bulb) do not elongate; their cells fail to divide, and elongate only slightly. See table 5 for cell behavior in coleoptiles of same seedlings.

The data obtained in a study of cell behavior in the light and dark-grown coleoptiles are shown also in table 5. In both the light and dark-grown plants, the coleoptile cell numbers increased markedly during the first twenty-four hours and the number had more than trebled at the end of forty-two hours. The Mazda illumination had comparatively little influence upon length of the coleoptile and behavior of its constituent cells in the early stages of germination. It seems to be a significant fact that light of low or moderate intensity inhibits growth of the internode, but at the same time exerts but little influence upon the coleoptile. The effect of very intense light upon early aging of the coleoptile has already been brought out.

Discussion

The first internode of grass seedlings grown in darkness undergoes marked elongation (polarized growth). Numerous factors have been suggested as the cause of this elongation, among them low temperature, low moisture content of the soil, and high CO_2 content of the atmosphere (**4**). Many years ago ROTHERT (**20**) pointed out that temporary illumination was effective in checking the development of this internode. Through the investigations of LANGE (**15, 16**), BEYER (**2**), DU BUY and NUERNBERGK (**9, 10, 11**), and HAMADA (**12, 13**), it has become clear that elongation of the *Avena* first internode may be suppressed by irradiation even for brief periods and with both long and short wavelengths of light. LANGE (**15**) pointed out that phototropically inactive red light, applied during the period of swelling and for twelve to sixteen hours after emergence of the coleoptile, inhibited elongation of the internode. HAMADA worked with the effect of light and temperature in relation to age of the seedling as factors concerned in internode shortening. The age at which maximum inhibition was brought about by light came earlier in germination, the higher the temperature. DU BUY (**8**) found that monochromatic blue and red light was very effective in shortening both internode and coleoptile. Elongation can be checked also by heat radiation (**10**). VAN OVERBEEK (**17**) has reported that exposure to the red plus infra-red radiation of an ordinary darkroom lamp brought about short internodes and decreased growth hormone con-

tent in the tips of *Avena*. It is known from the work of several investigators (**4, 7, 8, 21**) that high intensities of radiation inactivate plant growth hormone; also that the hormone is a limiting factor in extension of the internode under conditions of exposure to light.

JOHNSTON (**14**) has found that internode elongation can be inhibited by exposure to extremely low intensities of red and blue light in which the plants are invisible to the human eye. The data reported here indicate that the effective radiant energy required for threshold inhibition is very low. Less than one erg/mm.^{2}/sec. was sufficient to bring about an appreciable effect, so it may be seen that the mechanism involved is extremely photosensitive.

DU BUY and NUERNBERGK (**11**) studied the number of cells in longitudinal sections of first internodes shortened by light and found that no cell division took place under the conditions of their experiments. The experiments reported here with different intensities of light show that the extent of cell division in the internode depends upon the amount of light. Below the intensity causing maximum inhibition, cell multiplication takes place to an extent which varies inversely with the intensity and almost directly with the final length of internode attained.

The gain in length of the first internode (grown in darkness) may be found from the data of table 5, where the average size in the embryo was 0.283 mm. and the final length 72.8 mm. The final length was 257 times the original length in the seed. Analysis of the cell behavior shows 8.5 times as many cells in the mature internode and an average cell length of 30.1 times that in the embryo. Further analysis shows that in strongly illuminated internodes the cells increase in average length to about 4.5 times their original size, or from 13.5 to 60.8 μ. The striking difference between cell behavior in darkness and light is in the cell division activity. In the light no division takes place, and hence the internodes remain at about the same length as that attained by the dark internodes in the swelling stage just prior to initiation of their cell divisions.

In a study of the influence of light and darkness upon cell behavior in the epicotyl of *Phaseolus multiflorus*, BROTHERTON and BARTLETT (**5**) concluded that light "retards extension of the cells, and that as

an indirect result there are fewer secondary divisions, since relatively fewer primary cells enter the range of length within which division takes place." PENFOUND (**18**) observed that the increased height of shade-grown *Helianthus* seedlings was accompanied by an increase in the number of cells, not increase in length of cells, in the longitudinal axis. A number of other investigators have found that the effect of light upon growth of organs and tissues is concerned with both cell division and cell extension (*cf.* BURKHOLDER, **6**).

Examination of longitudinal sections through *Avena* embryos in early stages of germination in both light and darkness has shown that during the uptake of water considerable swelling occurs, accompanied by enlargement of the cells. In strong light the cells of the internode reach an average length of 35.9 μ at the end of thirty-six hours. At this time the cells of the darkened plants are beginning to divide; their average size is 26.2 μ. Measurements of cells which had been in division stages in darkness yielded length values of 28 and 36 μ at thirty hours of age.

The cells of illuminated internodes increase in size just as do those in darkness during early germination, but they do not divide. Their further enlargement is negligible; the internode fails to grow. It is clear that in early seedling growth light inhibits cell division in the internode; this is not an indirect influence brought about through failure of the cells to enlarge. The influencing factors are probably concerned with certain substances necessary for cell division, and if so, such substances must be rendered ineffective, or are changed in their path of movement, by very low intensities of light.

Summary

1. Twenty varieties of three species of *Avena* were germinated in complete darkness and with preliminary light treatment in the early stages of soaking, followed by growth in darkness. The ratio of internode length to coleoptile length differed markedly in the different varieties.

2. Final length of the first internode of *Avena sativa* var. Victory grown under a series of different intensities of weak Mazda light

varied inversely with the intensity. Similar inhibition of internode elongation in proportion to the intensity of Neon light was found at intensities below 0.1 erg/mm.^{2}/sec.

3. Early stages of germination in Victory oats in light (1000 watt Mazda) and in darkness showed that the internode elongated slightly during early swelling (up to thirty-six hours) in both light and darkness. In darkness further polarized growth occurred in the internode, but in strong light growth ceased early and shifted to the coleoptile.

4. Different amounts of light influenced polarized growth in different organs and tissues in different ways. Very low intensities of light inhibited growth of the first internode but not that of the coleoptile; high intensities inhibited the internode and appreciably shortened the coleoptile (by decreasing the number of cells as well as cell length). In complete absence of light the internode grew extensively, and the coleoptile was somewhat shorter than in plants which received small amounts of light in the early stages of germination.

5. Analyses of cell behavior in the first internode when grown to maturity under different intensities of Mazda light indicated that both cell division and cell enlargement were responsible for polarized growth.

6. During early germination, cell enlargement (up to a certain size) was found to occur in the first internode whether the seedlings were grown in light or darkness; in later development, cell division occurred in darkness and under very low intensities but not in bright light. The size of dividing cells during early germination in darkness was somewhat less than that attained by the cells of internodes grown in light.

7. In darkness, both cell division and cell elongation contributed to growth of the internode in length. The number of internode cells increased from embryo to maturity by 8.5 times and the average cell length increased by 30 times.

8. The effect of light in shortening the first internode of the axis was brought about primarily by inhibition of cell division. It is suggested that the influencing factors are probably concerned with cer-

tain substances necessary for cell division, and if so, such substances must be rendered ineffective, or are changed in their path of movement, by very low intensities of light.

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CULTIVATION IN VITRO OF EXCISED PEA ROOTS

JAMES BONNER AND FRED ADDICOTT

(WITH THREE FIGURES)

Introduction

There are many complicated interactions between the individual parts of the developing organism. That these interactions are due, at least in part, to the production, transport, and action of particular chemical substances has perhaps been earlier only an hypothesis. The work of the past few years has shown, however, that some of the effects of one plant organ upon the development of another are to be ascribed to the substance auxin, a typical hormone, which is produced in particular regions of the plant and influences various processes, notably cell enlargement, in other regions. Auxin is not the only specific substance or factor involved in the process of development. In fact, auxin acts only in conjunction with other, as yet unknown, factors in the initiation of roots, the formation of swellings, etc. (**25**). It was with the hope of isolating and identifying still other substances concerned with growth and differentiation that the present work was undertaken.

HABERLANDT (**9**) was the first to suggest that the reciprocal relationships of cells and organs might well be studied through observation of the results obtained by severance of these relationships; that is, by cultivation of isolated cells and organs in vitro. The present paper is concerned with a problem of this kind, namely, the cultivation in vitro of plant roots.

The first relatively successful cultures of isolated root meristems were those of ROBBINS (**17, 18**), ROBBINS and MANEVAL (**19**), and KOTTE (**12, 13**). These workers cultivated tips of *Pisum* and of *Zea* roots in various media and established the important fact that an excised root meristem, if it grows, continues to develop as a normal root. Differentiation into the usual root tissues takes place and secondaries may even be formed. Moreover, both KOTTE and ROBBINS found that addition of extracts to the culture medium may some-

what improve the growth of isolated roots. ROBBINS and ROBBINS and MANEVAL used yeast autolyzate for this purpose. KOTTE obtained beneficial effects with meat extract. ROBBINS also attempted to subculture corn roots. Short tips were cut from the cultured root after it had grown to several times its original length, and these fresh tips were placed in fresh medium and allowed to grow again. This procedure was then repeated through several transfers or passages, and it was found that the growth rate decreased rapidly in successive transfers and that the roots eventually died.

WHITE (**26, 27, 28**) undertook an extensive series of investigations into the optimum conditions for the cultivation of excised roots. In 1934 he was able to announce the "potentially unlimited growth" of tomato roots in vitro (**29**). Tomato roots, transferred weekly and having an average growth rate of 6.2 mm. per day, were kept in culture for four years (**32**). The medium which WHITE used contained in addition to inorganic salts and sucrose 0.01 per cent of yeast extract. Since the yeast extract is essential for the growth of these roots, it must contain accessory growth factors which the root is unable to synthesize for itself.

Studies of the cultivation of roots in vitro have also been made by GAUTHERET (**7**), DAUPHINÉ (**3, 4**), CHAMBERS (**2**), GEIGER-HUBER and BURLET (**8**), LOO and LOO (**14**), FIEDLER (**6**), and others.

Material and methods

Roots of *Pisum sativum* were employed in all the cultures. Attempts to culture these roots have previously been made by KOTTE (**12, 13**), ROBBINS, BARTLEY, and WHITE (**20**), and FIEDLER (**6**), but in no case have strikingly good results been obtained, nor has an extensive investigation of cultural conditions been made. The present work therefore began with a systematic inquiry as to the influence of several environmental factors and a search for a suitable culture medium.

Seeds of an inbred strain of peas, Perfection, kindly supplied by the Ferry-Morse Seed Company, were used for all of the later experiments. For the earlier tests seeds of Alaska and Perfection strains of the Moscow (Idaho) Seed Company were employed. The seeds were washed briefly with 95 per cent alcohol to remove a por-

tion of the fatty covering of the seed coat, sterilized in 0.1 per cent $HgCl_2$ for twenty minutes, washed with sterile water, transferred to sterile petri dishes containing a small amount of sterile water, and allowed to germinate for forty-eight hours. At the end of this time the roots were 5–10 mm. long. A portion of the apical meristem (including the root cap) 3–4 mm. long was then excised with a surgical scalpel (Bard-Parker, no. 7 handle with no. 10 blade). Such knives were found to be more satisfactory and more convenient than scissors, fragments of razor blades, or ground sewing needles (**20**), and less expensive than cataract knives. Transfer of the root tips was accomplished with a fine pair of forceps, great care being taken to avoid injury to the meristems.

All cultures were carried out in a small room built for the purpose. The walls, table, and floor of this room were washed periodically with a dilute solution of Lysol. Immediately before the room was used it was washed out with a fine water spray which thoroughly removed bacteria and mold spores from the atmosphere. The culture table was then covered with a freshly sterilized cloth. Knives and forceps were sterilized in alcohol and flamed before using. Infections occurring during the culturing were extremely rare.

The cultures were kept in a darkened cabinet at room temperature. This entailed regrettable but unavoidable fluctuations of temperature with corresponding fluctuations in the growth rate of the roots, even though an attempt was made to keep the room as close to 23°–25° C. as possible. Table 1 gives the results of one experiment in which roots were cultured at three different temperatures under otherwise similar conditions, and shows the magnitude of the variations to be expected from this source.

A comparison of various methods of dishwashing was also made. It was found completely satisfactory to wash the culture vessels in sulphuric acid-dichromate cleaning fluid, followed by rinsing in tap and distilled water. This method gave better growth of the roots than washing the vessels with soap, and was not improved by other modifications such as additional rinsing with hot water.

In early cultures the growth increments were small (5–9 mm. per week) and the roots appeared definitely unhealthy, as judged by the frequent brown discoloration. This was due primarily to the fact

that ordinary laboratory distilled water had been used in making up the medium. Only when water redistilled from pyrex was used could regular and extensive growth be obtained.

Before a standard culture technique was finally decided upon, various types of culture vessels were investigated, namely 50 and 125 cc. Erlenmeyer flasks and 10 cm. petri dishes. When liquid medium was used in the flasks growth was irregular and extremely poor, in marked contrast to the behavior of tomato roots which grow excellently under such conditions (**29**). Agar medium in flasks was somewhat more satisfactory, but the best results were obtained with petri dishes. In these vessels root growth was as good on liquid as

TABLE 1

EFFECT OF TEMPERATURE ON GROWTH OF EXCISED PEA ROOTS

TEMPERATURE	GROWTH IN MM. IN ONE WEEK
17° C.	18.2±1.0
24	26.4±1.8
27	29.3±2.2

on agar medium. FIEDLER (**6**) has recently demonstrated the importance of adequate aeration for the growth of excised roots, and it seems probable that the success of the petri dish as a culture vessel is due to the fact that the medium is spread out in a relatively thin, well aerated layer. Ten cm. petri dishes, charged with 15 cc. of the medium under investigation, were therefore adopted as the standard culture vessels for the further experiments. Two roots were grown in each dish.

Investigation

I. EXPERIMENTS ON FRESHLY EXCISED ROOTS

In any work of this kind, in which the cultural conditions are of great importance, the optimal conditions can be arrived at only by a series of successive approximations. For example, the iron concentration in the medium may be improved until some other factor becomes limiting. This second condition or factor may then be improved and further work upon the first factor thus made possible.

The present investigation was necessarily conducted in this way. For the sake of clearness, however, the results are not presented chronologically; so far as possible the experiments upon each culture condition are grouped together.

EFFECT OF LENGTH OF INITIAL TIP.—In order to ascertain the most favorable size of the initial fragment, tips of different lengths were cultured in the medium whose composition is shown in table 3. Table 2 gives the result of one such experiment. Tips having a length of less than 0.5 mm. very rarely developed into normal roots. In most cases the fragment either elongated slightly and then ceased

TABLE 2

EFFECT OF LENGTH OF INITIAL ROOT TIP UPON GROWTH OF EXCISED PEA ROOTS

LENGTH OF TIP (MM.)	PERCENTAGE OF TIPS WHICH GREW AS NORMAL ROOTS	AVERAGE LENGTH OF NORMAL ROOTS
0.5	0	.....
1.0	18	7.4
2.0	50	25.8
3.0	100	44.0
4.0	100	44.3

growth completely, or developed into a colony of loose parenchymatous cells similar to those described by ROBBINS, BARTLEY, and WHITE (**20**) for very short corn root tips. In neither case was any trace of vascular tissue formed. Tips 0.5–1.0 mm. in length developed into normal roots in about 20 per cent of the cases. The growth rate of roots from these short fragments, however, was considerably slower than that of roots taken from longer ones. If tips greater than 3 mm. were taken, all of them grew as normal roots and their growth rate was unaffected by further increase of the initial length. Measurements upon sectioned roots similar to those used in the cultures showed that the distance from the apical end to the common initial zone is about 0.45 mm. The initial zone itself is less than 0.1 mm. in length. It is therefore clear that for normal growth of the root a considerable portion in addition to the initial zone is necessary. In particular, the development of vascular tissue must depend upon

the presence in the tip of well defined prospective vascular tissue, since such tissue was not formed by tips containing only the initial zone. The presence of the differentiating histogen zone alone was not sufficient for normal growth, however. If a 0.5 mm. fragment was removed from the root, normal growth of the following portion did not take place even if this portion was as long as 2 mm. Such fragments merely elongated for a brief period and then stopped growth completely. For the production of a normal root both the initial zone and a relatively large amount of the following portion are necessary. For all of the subsequent cultures tips having a length of 3–4 mm. were therefore used.

TABLE 3

INORGANIC CONSTITUENTS OF MEDIUM USED FOR CULTIVATION OF EXCISED PEA ROOTS

SUBSTANCE	WHITE'S MEDIUM MG./LITER	PEA ROOT MEDIUM MG./LITER
$Ca(NO_3)_2 \cdot 4\ H_2O$	142	236
$MgSO_4 \cdot 7\ H_2O$	74	36
KNO_3	81	81
KCl	65	65
KH_2PO_4	12	12
$Fe_2(SO_4)_3$ or	2.5	2
Ferric tartrate		1

INORGANIC CONSTITUENTS OF MEDIUM.—For the first experiments, which were directed toward the satisfactory culture of freshly excised roots, a medium was chosen having the composition of that used by WHITE (**29**) for the culture of tomato roots. The composition of this medium is given in table 3. Two per cent glucose was used as a carbohydrate source. In this medium the freshly excised roots grew regularly 25–30 mm. during the first week. Additions of yeast extract, peptone, boron, manganese, and zinc, each over a wide range of concentrations, as well as variation of the glucose concentration, had little effect.

Growth was clearly limited by some factor or condition other than those investigated. There were of course a great number of possibilities. WHITE (**28**) has stressed the importance of the correct ionic composition of the medium, and it was therefore decided to investi-

gate the effects of varying the concentration of each salt. The roots were grown for one week under the standard cultural conditions just outlined. A medium having the composition shown in table 3 was used as a basis for the variations, since, as will be shown, this medium is on the whole the best of those tried. In each series one of the salts

TABLE 4

INFLUENCE OF IONIC MILIEU ON GROWTH OF EXCISED PEA ROOTS IN 2 PER CENT GLUCOSE MEDIUM

Relative concentration of salt	Growth in mm. in one week		
	$Ca(NO_3)_2 \cdot 4H_2O$	$MgSO_4 \cdot 7H_2O$	KNO_3
	1X=236 mg./l.	1X=36 mg./l.	1X=81 mg./l.
4X	22.3±1.8	22.4±1.1	20.2±1.4
2	19.0±2.4	24.0±0.9	19.5±2.2
1	30.0±1.2	25.5±0.9	23.5±0.6
0.5	21.8±1.5	24.6±1.0	22.9±1.0
0.25X	22.7±1.6	24.6±1.2	21.5±1.7

Relative concentration of salt	Growth in mm. in one week		
	KCl	KH_2PO_4	NH_4NO_3
	1X=65 mg./l.	1X=12 mg./l.	1X=1250 mg./l.
4X	23.7±1.1	32.9±1.6	23.0±1.2
2	27.2±0.9	28.5±1.0	27.5±1.2
1	28.5±1.5	30.6±1.5	31.1±1.5
0.5	27.8±0.7	27.5±1.4	23.2±1.4
0.25X	23.3±1.1	28.4±1.4	27.0±1.9

was used in concentrations of 4, 2, 1, 0.5, and 0.25 of its concentration in the basic medium. The results from a number of experiments are presented in table 4, together with the probable errors of each determination. These probable errors will suffice to give an indication of the variation to be expected when ten to twelve roots are used. Included in table 4 also is a series in which NH_4NO_3 replaced the KNO_3. It is evident that within the concentration limits considered,

the inorganic composition of the medium has little effect upon the growth of these roots. The substitution of ammonium for nitrate as a source of nitrogen is also without beneficial effect. One can conclude only that other factors are limiting the growth.

Various concentrations of iron in the form of $Fe_2(SO_4)_3$ were used (table 5). Concentrations of 4 and 5 mg. per liter were markedly toxic, but the lower concentrations all appeared to have about the

TABLE 5

INFLUENCE OF IRON CONCENTRATION UPON GROWTH OF EXCISED PEA ROOTS

$Fe_2(SO_4)_3$ mg./liter	0.25	0.5	1.0	2.0	4.0	5.0
Growth in mm. in one week	27	26	28	27	19	8

TABLE 6

COMPARISON OF TARTRATE AND SULPHATE AS IRON SOURCE FOR GROWTH OF EXCISED PEA ROOTS

	Growth in mm. in one week		
	1 mg./liter	2 mg./liter	3 mg./liter
Ferric sulphate	26	26	27
Ferric tartrate	27	26	25

same effect. It was possible that sources of iron other than the sulphate might be more favorable. Ferric chloride, ferric phosphate, ferric sulphate, and ferrous and ferric tartrate were therefore compared. It was found that when glucose was used as the carbohydrate source the tartrate was markedly more favorable. This difference disappeared, as is shown in table 6, when conditions were improved by the use of sucrose as the source of carbohydrate, and need not be gone into in detail.

A second series of experiments was run in which 2 per cent sucrose rather than 2 per cent glucose was used as the carbohydrate and in which the inorganic constituents of the medium were varied as in table 4. As is shown later, sucrose is superior to glucose for the pea root. In this series the variations of growth increment with changes

in the ionic composition of the medium were even smaller than in the first series. It is clear that under the conditions thus far examined the relative amounts of the various inorganic constituents of the medium are not of great significance in determining the growth of excised pea roots. The medium whose composition is given in table 3 is on the whole somewhat superior to those of the other compositions tried, and it was therefore used in all of the later experiments. It may well be, however, that when still other limiting factors are removed (see later) the ionic nature of the medium is of more importance.

TYPE OF CARBOHYDRATE.—There had been some indications that different sugars differed in their capacity to support the growth of pea roots. This is not surprising, since ROBBINS and WHITE (**22**) have shown that for *Zea* roots dextrose is superior to fructose, xylose, sucrose, or maltose. On the other hand WHITE (**30**) has found that sucrose is superior to glucose for tomato roots. A comparison between a number of sugars was therefore made. Baker c.p. glucose, Merck c.p. glucose, Pfanstiehl c.p. glucose (for injection), Pfanstiehl c.p. fructose, Pfanstiehl c.p. galatose, and Baker c.p. sucrose were used, each in 2 per cent concentration. The results of one experiment are shown in table 7. Galactose is used little if at all and the growth is slight. The three glucose preparations are much better and are essentially equal among themselves. Fructose is slightly better than glucose, but the Baker sucrose is definitely the best of all. This difference between glucose and sucrose as a carbohydrate source for pea roots has been found consistently in many experiments, but the reason for it is still obscure. It may be that the readily crystallizable sucrose contains less of some heavy metal impurity than does the glucose, or there may be some metabolic basis for the difference. It is of interest that pea roots resemble tomato roots in that they prefer sucrose to glucose, whereas the two grass roots which have been studied extensively (wheat and corn) definitely prefer glucose to sucrose.

CONCENTRATION OF CARBOHYDRATE.—The use of 2 per cent sugar has apparently become traditional among those who attempt the cultivation of excised roots. ROBBINS and MANEVAL (**19**), however, pointed out many years ago that growth in length, increase of dry

weight, and number of secondaries produced by excised *Zea* roots is greater in 4 per cent glucose medium than in 2 per cent. MALYSCHEV (15) apparently used sugars in concentrations other than 2 per cent, but his findings are not reported in detail. WHITE has used 2 per cent sugar, as have GAUTHERET (7), ROBBINS and coworkers (20, 21, 22, 23), LOO and LOO (14), FIEDLER (6), and others. There have been occasional brief departures from the tradition (13, 8), but the more extensive investigations have in general employed sugar in the concentration of 2 per cent. At the beginning of the present work different glucose concentrations were compared, but

TABLE 7

COMPARISON OF VARIOUS SUGARS AS CARBOHYDRATE SOURCE FOR GROWTH OF EXCISED PEA ROOTS; EACH SUGAR USED IN 2 PER CENT CONCENTRATION

SUGAR	GROWTH IN MM. IN ONE WEEK
Glucose (Merck)	22.5
Glucose (Pfanstiehl)	22.0
Glucose (Baker)	22.3
Fructose (Pfanstiehl)	25.5
Galactose (Pfanstiehl)	5.3
Sucrose (Baker)	33.0

because of the small and variable growth of these early cultures, of course due to the fact that other conditions were at that time limiting, no obvious effect was observed. Different concentrations of sucrose were therefore tried, using the improved medium. A series of typical results is presented in table 8. In the absence of sugar the tips grew very little, and what growth did take place was undoubtedly to be attributed to sugar stored in the initial fragment. With increasing sucrose concentration the growth increment increased rapidly until 4 per cent was reached. Further increases were then without effect. It is clear that under the conditions used in these experiments growth is limited by sugar concentration unless the latter is 4 per cent or more. The fact that the earlier sucrose cultures were limited by the low sugar concentration may partially explain their lack of response to changes in the ionic milieu and to special factors.

The growth of 3–4 mm. tips in one week in the standard medium with 4 per cent sucrose has varied between 60 and 95 mm. in the many series which have since been made. The growth of similar roots, under the same conditions but attached to the seed, is about 75 mm. in the first week. The excised roots may therefore be considered as normal in so far as the growth rate is concerned.

INFLUENCE OF SPECIAL FACTORS.—Throughout the course of the present work attention was directed toward the various accessory substances or special substances which one might suppose the seedling root normally to receive from the cotyledons. It will be shown

TABLE 8

EFFECT OF SUCROSE CONCENTRATION UPON GROWTH OF EXCISED PEA ROOTS

Percentage sucrose concentration	0	0.5	1.0	2.0	3.0	4.0	5.0	6.0
Growth in mm. in one week	7	20	29	33	43	56	57	55

in section II that the influence of such factors can be much better studied in roots which have been in culture for some time and in which the supply present in the freshly excised tip has been depleted. Nevertheless for comparison with the work of others it will be of interest to present a few of the results obtained with freshly excised roots. The experiments unless otherwise noted were run with the standard medium and 2 per cent carbohydrate.

The first and obvious experiment was to place pea cotyledons (with the embryo removed) face downward in the petri dish containing roots. Substances necessary for the growth of roots could then diffuse out of the cotyledon and into the medium, whence they might be taken up by the growing root. As shown in table 9, however, living cotyledons give up substances which are inhibitory to the growth of roots, and dead cotyledons give up even more. Recourse was therefore had to peptone and to yeast extract.[1] Peptone definitely

[1] Yeast extract was made by boiling 2 gm. of Fleischman's dry brewers' yeast for 20 minutes, filtering, making up to volume, and diluting with medium to the desired concentration. The concentration of yeast extract is expressed in terms of the original dry weight of yeast. Thus 0.01 yeast extract is the extract of 0.01 gm. of dry yeast in 100 cc. of medium.

increased the growth increment (table 10). The roots receiving 0.08 per cent peptone grew in this case 50 per cent better than the controls. Yeast extract also had a small beneficial effect (table 10), amounting to about 20 per cent for a concentration of 0.02 per cent. Although the improvement in growth resulting from the addition of peptone is marked, and even that resulting from the addition of

TABLE 9

INFLUENCE OF COTYLEDONS UPON GROWTH OF EXCISED PEA ROOTS

	CONTROL WITHOUT ADDITION	FRESH COTYLEDONS ADDED	AUTOCLAVED COTYLEDONS ADDED
Growth in mm. in one week	37	28	21

TABLE 10

INFLUENCE OF PEPTONE AND YEAST EXTRACT UPON GROWTH OF EXCISED PEA ROOTS IN 2 PER CENT SUGAR MEDIUM

Percentage peptone concentration	0	0.001	0.005	0.01	0.02	0.04	0.08
Growth in mm. in one week	28	29	33	34	35	36	42
Percentage yeast extract concentration	0	.....	.....	.....	0.02	0.04	0.08
Growth in mm. in one week	30	.....	.....	.....	37	35	33

yeast extract is considerable, neither is spectacular. What effect is present disappears upon still further improvement of the medium, as will be shown later. In part II it will be shown, however, that under the correct conditions both of these crude preparations do possess growth promoting activity.

It was thought that asparagin might prove to be a better source of nitrogen alone. Table 11 shows that it is little if any better. This result might perhaps have been expected since, as has been demonstrated by MCKIE and BARNETT (**16**), the pea seedling is a typical "amino acid" rather than an "asparagin" plant; that is, in the nitrogen metabolism of the pea seedling amino acids rather than asparagin are the chief translocation forms of nitrogen.

Inositol, the well known bios I of EASTCOTT (5), is also without effect upon the growth of pea roots (table 11). Cysteine is markedly inhibitory in concentrations of 10 mg. per liter or more, but seems to have some beneficial effect in lower concentrations. The role of cysteine has not been investigated further.

Root tips grown in 4 per cent sucrose medium do not respond to added yeast extract as do those grown in 2 per cent sucrose medium, as may be seen in table 12. The effect found in the low carbohydrate

TABLE 11

INFLUENCE OF ASPARAGIN AND OF INOSITOL UPON GROWTH OF EXCISED PEA ROOTS

Asparagin concentration....	0	1–5 mg./liter	15–50 mg./liter
Growth in mm. in one week	30	33	32
Inositol concentration.....	0	1 mg./liter	10 mg./liter
Growth in mm. in one week	31	33	31

TABLE 12

EFFECT OF YEAST EXTRACT UPON GROWTH OF EXCISED PEA ROOTS IN 4 PER CENT SUCROSE MEDIUM

Percentage yeast extract concentration.....	0	0.005	0.02	0.08
Growth in mm. in one week.....	75.5	73.5	70.5	64.5

medium may therefore be of a purely nutrient nature. This view is supported by the fact that the highest yeast concentration, 0.08 per cent, is markedly inhibitory in the 4 per cent sucrose medium whereas it is distinctly beneficial in the medium containing only 2 per cent sucrose. It will be shown in section II that yeast extract does contain substances necessary for the continued growth of pea roots. The initial fragment, however, seems to contain sufficient of these substances for a considerable growth if other circumstances are favorable. Essentially the same conclusion has been arrived at by ROBBINS (18) for corn roots.

II. SUBCULTURE OF EXCISED PEA ROOTS

In part I roots grown from freshly excised tips were considered exclusively. Attention will now be turned to the subculturing of

these roots. Transfers (or subcultures) were carried out in essentially the same manner as that described in part I. The same medium containing 4 per cent sucrose was used in 10 cm. petri dishes, twenty to forty roots being used for each part of each experiment. Cultures were again kept in a darkened cabinet and were subject to the same temperature fluctuations. Transfers, unless otherwise specified, were made at approximately weekly intervals.

INFLUENCE OF YEAST EXTRACT.—Tips 1 cm. long were cut from roots grown from freshly excised fragments and were transferred again to the standard medium. At the end of the second week this was repeated, and again after the third week. Table 13 gives the

TABLE 13

SUBCULTURE OF EXCISED PEA ROOTS IN BASIC MEDIUM WITHOUT ADDITION OF ACCESSORY FACTORS

EXPERIMENT NO.	GROWTH IN MM. PER PASSAGE			
	1	2	3	4
1	65	11	3	0
2	63	11	4	0
3	75	11	0	0
4	59	10	0	0

results of four typical experiments. Passage 1 represents the freshly excised roots; passage 2, the second week; and so on. The growth rate drops very rapidly and becomes practically nil in the third passage. One would first suppose, as ROBBINS (**18**) did originally, that a substance (or substances) necessary for growth is present in the initial tip in quantities sufficient for the first passage but not for the later ones. This is undoubtedly true in part, but the situation is still somewhat more complicated, as is shown by the following experiment. A large number of freshly excised tips were cultured for one week (passage 1). At the end of this time, when the roots had grown 59 mm., one half of them were subcultured into fresh medium and one half were allowed to remain undisturbed. At the end of the second week the growth increment in passage 1 plus that in passage 2, or the total growth of the transferred roots, was 69 mm.; that of the undisturbed roots was 201 mm. There must then have been

sufficient growth factor present in the initial fragment to permit much more growth than was obtained in the transferred roots. ROBBINS (18) has suggested that the growth factors may be fractionated in some unspecified manner by the process of subculture. Another possibility is that a portion of the active substances is destroyed by the enzymes set free at each new cut surface. Which of these or other possible explanations is the correct one, is as yet unknown.

It has already been pointed out that in the 4 per cent sucrose medium yeast extract has no beneficial effect upon the growth of the initial passage. In marked contrast to this is the effect of yeast extract on the later passages. Table 14 shows the result of an experi-

TABLE 14

SUBCULTURE OF EXCISED PEA ROOTS IN MEDIUM CONTAINING 0.02 PER CENT YEAST EXTRACT

	GROWTH IN MM. PER PASSAGE							
	1	2	3	4	5	6	7	8
No yeast extract.....	59	10	0	0	0	0	0	0
0.02% yeast extract transferred weekly.....	58	33	36	42	54	47	44	57
	(1 & 2)		(3 & 4)		(5 & 6)		(7 & 8)	
0.02% yeast extract transferred each two weeks..	155		112		140		265	

ment in which one series was transferred weekly in medium containing no yeast extract, one series weekly in medium containing 0.02 per cent yeast, and one series every two weeks in yeast medium of the same concentration. As usual, the roots cultured in ordinary medium finished their growth in the third passage. Those in yeast extract on the other hand grew 33 mm. in the second passage but recovered to an average of about 50 mm. per passage for the last six passages. Those roots which were transferred only once in two weeks showed more irregular results but continued to grow excellently.

The total growth increment of the two week roots is again considerably larger than that of the one week roots.

Extensive experiments with different concentrations of yeast extract showed that for long continued subculture, 0.01 per cent yeast

extract is considerably superior to either 0.02 or 0.005 per cent. Roots were therefore cultivated in large numbers in the standard medium with the addition of 0.01 per cent yeast extract. Figure 1 shows the growth per passage of a series which was kept in culture for four months. At the end of this period the roots were perfectly normal and healthy in all respects and their growth rate was quite as high as at the beginning of the period. There seems every reason to

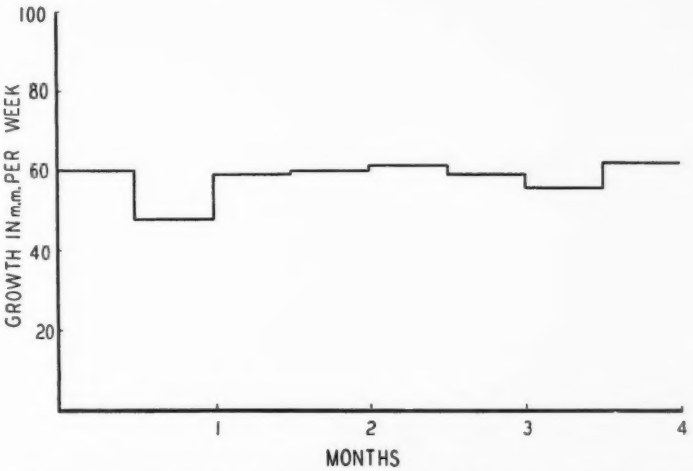

FIG. 1.—Growth rate of excised pea roots in vitro. Cultures transferred weekly in 4 per cent sucrose medium containing 0.01 per cent yeast extract. Means of successive pairs of passages.

suppose that these roots might have been kept in culture indefinitely, but it was necessary to discontinue the series for other reasons. Heretofore WHITE, and recently ROBBINS and BARTLEY (**23**), have succeeded in keeping tomato roots through several passages without decrease of growth rate. ROBBINS and coworkers (**18, 22**) have not been able to subculture *Zea* roots indefinitely, and FIEDLER (**6**) has concluded that for *Zea* roots this is impossible. It seems highly doubtful whether FIEDLER'S conclusion is justified. The less satisfactory media used in the early portions of this work (see part I) were incapable of supporting continued growth of pea roots. Only

when a satisfactory medium was found was continued growth possible. It seems likely therefore that the most suitable medium for the cultivation of roots such as those of *Zea* has not yet been found.

ACTIVE PRINCIPLES OF YEAST EXTRACT: VITAMIN B_1.—A systematic search for the active principles of yeast extract; that is, the substances in yeast extract which are necessary for root growth, was next attempted. At the time the investigation was begun there was no indication as to the possible nature of these substances. During the course of the work, however, several papers bearing upon the subject appeared (**21, 23, 22, 31, 1**). These will be discussed in connection with the work reported here.

Preliminary experiments indicated that a concentrate of vitamins B_1 and B_2 (Squibb) is considerably more effective than is yeast extract in promoting growth of the later passages of pea roots. Crystalline vitamin B_1, both the natural and the synthetic product (Merck),[2] was therefore investigated as to its possible accessory growth factor activity. No difference between the natural and the synthetic product was found, and both were highly active, as shown by the following experiment. Freshly excised root tips were cultivated in the standard medium for one week. Since no accessory factors were added, the root's own supply should be depleted and this should allow of a greater response in passage 2. The medium for the second passage consisted of the basic medium plus vitamin B_1 in concentration varying from 20 gamma per cc.[3] to 0.00002 gamma per cc. Controls with 0.01 per cent yeast extract and with no addition were run at the same time. Twenty roots were grown in each set. Table 15 gives the result of a typical experiment. The controls with no addition grew only a small amount as usual, 11 mm. Those with B_1 concentrations greater than 0.0002 gamma per cc. grew even better than the yeast extract controls. A maximum response was attained with 0.0002 to 0.002 gamma per cc., but even 0.00002 gamma per cc. sufficed to elicit a considerable growth response. It is not surprising that pure vitamin B_1 should be able to bring about more growth than is the optimum concentration of yeast extract, since as

[2] We are greatly indebted to the Merck Company for its cooperation in supplying vitamin B_1.

[3] One gamma is 10^{-6} gm.

has already been shown, yeast contains not only beneficial substances but detrimental ones also.

The minimum concentration of vitamin B_1 which is effective in promoting the growth of these roots is about 1–2 in 10^{11}. This activity is of the same order as that found by KÖGL and TÖNNIS (**10**) for the effect of "biotin" in promoting the growth of yeast. Maximum effectiveness is attained with a concentration of about 1 in 10^9, but no toxic effects have been observed with a concentration of many times this. In later experiments a concentration of 1–2 in 10^7 has

TABLE 15

EFFECT OF CRYSTALLINE VITAMIN B_1 UPON GROWTH OF EXCISED PEA ROOTS IN SECOND PASSAGE

B_1 concentration yeast/cc.	0	0.00002	0.0002	0.002	0.02	0.2	2.0	20.0	0.01% yeast extract
Growth in mm. in one week	11	40	64	70	70	62	68	61	48

regularly been used, since it was desired to leave an adequate margin to allow for destruction, etc.

Crystalline vitamin B_2 (lactoflavin)[4] was also tested for its activity in promoting the growth of pea roots. The sample used did possess some activity, although several thousand times less than that of B_1. Roots grown with B_2 also never attained the luxuriance of the B_1 roots. It may be that activity of the sample used (a natural product) was merely the result of minute amounts of B_1 present as an impurity. This hypothesis is supported by the fact that added B_2 did not augment the effect of B_1.

Simultaneously with and independent of the work presented here (and in a preliminary form in an earlier paper, **1**), it was found by ROBBINS and BARTLEY (**23**) that vitamin B_1 is an essential factor for the continued growth of excised tomato roots. The minimum concentration which they found to be effective is of the same order as that found for pea roots. In an earlier paper, however, ROBBINS

[4] The lactoflavin preparation was kindly supplied by Professor LEPKOVSKY, University of California.

and WHITE (**22**) found that vitamin B (presumably B_1) is without effect on the growth of freshly excised *Zea* roots. While a lack of effect upon the freshly excised root would not be surprising, work in this laboratory (unpublished) has indicated that even roots attached to the seed may benefit from additions of B_1.

It is perhaps well to point out that there is a physiological function for the vitamin B_1 which is normally stored in seeds (**24**), a

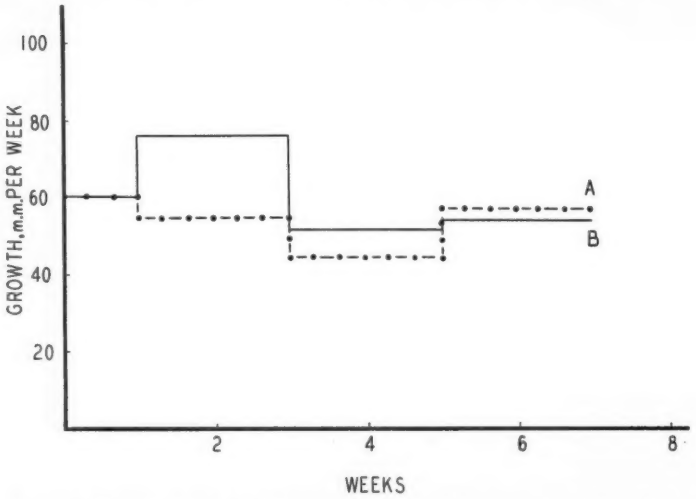

FIG. 2.—Growth rate of excised pea roots in vitro. Cultures transferred weekly in *A:* medium containing 0.01 per cent yeast extract; *B:* medium containing 1 in 10^7 parts of vitamin B_1, as accessory growth factors. Means of successive pairs of passages.

conclusion which has been independently arrived at in a different manner by KÖGL and HAAGEN-SMIT (**11**).

ACTIVE PRINCIPLES OF YEAST EXTRACT: AMINO ACIDS.—Pea roots may be carried through several passages in basic medium containing vitamin B_1 as the only accessory factor. This has been indicated in a previous publication (**1**) and a further example is given in figure 2. During the first four passages the B_1 roots grew better than the controls with yeast extract. After this time, however, growth of the B_1 cultures lagged behind that of the controls and the roots became thin and abnormal in appearance. Such roots nevertheless may be

cultivated for a very long time (fig. 3). This series was transferred weekly for three and one half months. In this case the growth rate became stabilized at about 40 mm. per transfer in the later passages. If this decrease in growth rate during continued subculture is actually due to a second substance or substances, it should be possible to bring about the drop sooner by the use of shorter tips for each transfer. The "dilution" of the second factor should then take place

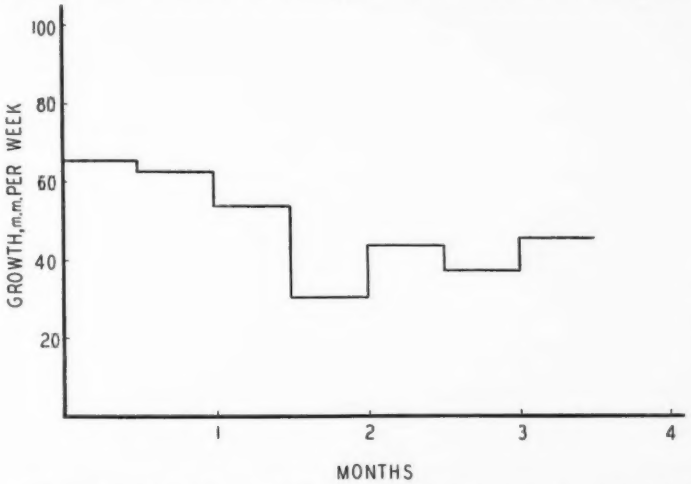

FIG. 3.—Growth rate of excised pea roots in vitro. Cultures transferred weekly in medium containing 1 in 10^7 parts of vitamin B_1 as accessory growth factor. Means of successive pairs of passages.

more rapidly. Table 16 shows that if 2 mm. tips are used instead of the usual 1 cm. ones, limitation of growth by the second factor does indeed become apparent in the third passage in B_1 medium. On the other hand, 2 mm. tips transferred each time to 0.01 per cent yeast extract medium grow normally although less rapidly than the 1 cm. tips. The individual variation among roots grown from 2 mm. tips, however, was so great as to make their use for further work impracticable. Five to 6 mm. tips were therefore employed for series in which it was desired to make the second factor limiting as soon as possible. With such tips this limitation becomes apparent in general in the fourth passage.

It was first thought that the second factor might be in the nature of inorganic material, for example metallic ions, present in the yeast extracts in small amounts. Yeast ash, however, did not improve in the slightest the growth of the later passages in B_1 medium. ROBBINS and coworkers (**21**) have shown that qualitative filter paper may act as a source of some apparently inorganic growth factor for *Zea* roots. Qualitative filter paper was also without effect as the second factor for pea roots. Other workers (**6, 8**) have found that indoleacetic acid in small concentrations may greatly increase the

TABLE 16

GROWTH OF SUBCULTURES OF EXCISED PEA ROOTS; TRANSFERS MADE WITH 2 MM. TIPS

	GROWTH IN MM. PER PASSAGE		
	2	3	4
Medium with B_1	43	14	8
Medium with yeast extract	38	27	38

growth of excised roots. It is true that their experiments have been done only with the initial passage, and in both cases with *Zea* roots. Indoleacetic acid has been used in the present cultures in concentrations of 10^{-10}, 10^{-11}, and 10^{-12} molal. These concentrations are of the order of those found to increase the growth of *Zea* roots. In the case of pea roots, however, no effect was found in passages 2 to 12.

It seemed possible that the second factor might merely be nitrogen in the amino form—that these roots might be unable to utilize indefinitely inorganic nitrogen alone, particularly since WHITE (**31**) has emphasized the importance of amino acids in root growth. Neither alanine nor natural leucine, however, possesses the slightest effect. Asparagin also was without beneficial influence over a wide range of concentrations. In marked contrast to the ineffectiveness of these single amino acids, Witte peptone was found to be quite effective as a supplement to vitamin B_1. This effect was obtained only over a narrow range of concentrations: 0.01 mg. peptone per cc. was almost without effect, 0.02 and 0.04 mg. per cc. were beneficial, and 0.1 mg. per cc. was markedly toxic. Even in the optimum peptone

concentration the roots in later passages appeared somewhat thicker than normal and were obviously unhealthy. The fact that peptone was active as a B_1 supplement, however, suggested that several amino acids might be needed; that the roots needed not amino nitrogen in general, but rather "essential" amino acids which they are unable to synthesize for themselves. A mixture of amino acids was therefore prepared. Each acid was crystalline and of as high quality as was obtainable. The following list indicates the acids used and their sources. We are greatly indebted to Professor H. BORSOOK for the supply of these amino acids.

glutamic acid hydrochloride	c. p. Pfanstiehl
d-l-alanine	Fox (made by S. Fox of these laboratories)
l-tyrosine	Fox (made by S. Fox of these laboratories)
d-arginine	Fox (made by S. Fox of these laboratories)
l-cysteine hydrochloride	Fox (made by S. Fox of these laboratories)
glycine	Fox (made by S. Fox of these laboratories)
cystine hydrochloride	Fox (made by S. Fox of these laboratories)
natural leucine	Fox (made by S. Fox of these laboratories)
d-l-serine	Amino acid made at the University of California at Los Angeles
d-l-methionine	Hoffman LaRoche
d-valine	Hoffman LaRoche
d-ornithine dihydrochloride	Hoffman LaRoche
d-lysine dihydrochloride	Hoffman LaRoche
l-histidine monohydrochloride	Hoffman LaRoche
trytophane	c. p. Pfanstiehl
asparagine	Merck

Equal amounts of each amino acid were weighed out, dissolved together, and various amounts of the mixture used either alone in the basic medium or as a supplement to vitamin B_1. With this mixture, as with peptone, the range of concentrations which could be used was narrow. It was found that 0.02 mg. per cc. was somewhat toxic;

0.002 mg. per cc. had little effect; but 0.01 mg. per cc. gave very satisfactory results (table 17). The vitamin B_1 series of this experiment grew, as usual, very well during the first three passages. In the fourth, fifth, and sixth passages it dropped to the lower growth rate characteristic of roots having B_1 as the only accessory factor.

The amino acid mixture alone was also beneficial but the roots became thin and were in poor condition at the end of the sixth passage. The combined effect of vitamin B_1 and the amino acid mixture was striking. These roots elongated rapidly during the entire six passages. They furthermore formed secondaries which the slower

TABLE 17

SUBCULTURE OF EXCISED PEA ROOTS; EFFECTS OF VITAMIN B_1 AND AN AMINO ACID MIXTURE, SINGLY AND TOGETHER

	GROWTH IN MM. PER PASSAGE				
	2	3	4	5	6
B_1 alone	58	68	35	34	36
Amino acid mixture alone	66	78	50	51	32
B_1 and amino acid mixture together	68	77	77	67	83

growing control series did not; they were of normal diameter and appeared to be in every way in the best of condition. It was unfortunately necessary for other reasons to interrupt this experiment after the sixth passage (and to terminate another series which had been through eight passages). Further experiments must therefore show whether pea roots may be subcultured for longer periods in this medium of completely known composition. There is at present every indication that they can be subcultured indefinitely. Roots grown in this medium are equal or superior to those grown in yeast extract, and there has been no sign of decreased growth rate in the later passages. Further experiments must also determine which of these amino acids are the essential ones, and the optimum concentration of each must be found.

That a complex mixture of amino acids, in addition to vitamin B_1, should be necessary for the continued growth of excised pea roots is not surprising. Amino acids are formed in large amounts in

the germinating pea (**16**) and they might be expected to possess some value for the seedling plant. The root of the mature plant also is "heterotrophic," in that it depends upon the green aerial portions for its supply of carbohydrates. One can easily imagine that there are also particular amino acids which the root is unable to synthesize and which must be supplied by the leaves. WHITE (**31**) has recently found that a complex mixture of amino acids is necessary for the growth in vitro of excised tomato roots. In his case also the amino acids act in conjunction with another factor which has not yet been identified but which from the work of ROBBINS and BARTLEY (**23**) and of BONNER (**1**) appears to be vitamin B_1.

Discussion

This investigation has shown that if a suitable medium is used, excised pea roots grow rapidly in length in vitro. Tips freshly excised from the plant grew, under the conditions used, 60 to 95 mm. in the first week, and formed one to four short lateral roots. Under less favorable conditions, for example, in the 2 per cent glucose medium rather than in the 4 per cent sucrose medium, growth in length was less and no secondaries were formed.

This lack of lateral formation in unsuitable media no doubt accounts for the fact that KOTTE (**13**), who obtained only 10–14 mm. growth in twelve days, never observed lateral formation on excised pea roots. If freshly excised tips were allowed to remain in the best medium for two (rather than one) weeks, the growth in length was 200 mm. or more, and as many as twenty-five laterals were formed on a single root. This secondary root formation also took place in the later passages. Roots giving good growth rates (in suitable medium containing 0.01 per cent yeast extract) formed one to four laterals in one week. The roots in the series of table 14 which were transferred only once in two weeks formed as many as twenty-five laterals per root in the fourth passage. Many attempts have been made to subculture these lateral growing points but without consistent success. An occasional lateral tip developed into a normal root, but the majority grew either slowly or not at all. Those which grew also remained thinner than normal.

This is an indication that even the best yeast extract medium is not optimal for the growth of excised pea roots, but further experi-

ments must determine what improvements can be made. Since it has been impossible to subculture the lateral growing points it has been impossible as yet to establish clones of pea roots, as has WHITE (29) with tomato roots.

It has long been known that crude extracts, and in particular yeast extract, are beneficial and even necessary for the growth of excised roots. Pea roots are no exception to this general rule. Of greater interest is the fact that it has been possible to substitute known chemical substances for the unknown "active principles" of yeast. Crystalline vitamin B_1 has been shown to be an accessory factor, a special chemical substance necessary, in minute amounts, for the growth of pea roots. As a complement to vitamin B_1, and necessary for continued growth, specific amino acids are also required in small amounts. Apparently it might equally well be said that vitamin B_1 acts as a complement to the specific amino acids. In any case, either alone is inadequate and only their combined activity will support the continued growth of pea roots. It has been said, concerning the cultivation of roots in vitro, that "The proved unlimited capacity for growth of such cultures obviously sets aside the concept of indispensable and *specific* correlation hormones" (30). As the writers view the matter, exactly the reverse is more obvious. Whether we call vitamin B_1 and the specific amino acids phyto-hormones or accessory growth substances, they are indispensable to the continued growth of these root cultures, and they must be, in normal plants, carriers of growth correlation between root and seed or shoot.

Summary

1. A medium suitable for the growth of excised pea roots has been described. This medium contains 4 per cent sucrose in addition to inorganic salts.

2. Freshly excised pea root tips 3–4 mm. long grow 60 to 95 mm. in this medium in the first week; 200 mm. or more in the first two weeks.

3. Yeast extract (0.01 per cent) must be added to the basic medium if good growth in passages after the first is to be obtained. Roots have been kept in culture with weekly transfers for four months on the basic medium with added yeast extract.

4. Crystalline vitamin B_1 is capable of partially replacing yeast

extract; vitamin B_1 alone is not able to support the continued optimal growth of excised pea roots in later passages.

5. A mixture of pure, crystalline amino acids is capable of replacing that portion of the activity of yeast extract which is not due to vitamin B_1. With the use of vitamin B_1 and this mixture of amino acids a highly satisfactory, completely known medium for the cultivation of excised pea roots has been obtained. This medium of known composition supports the growth of such roots as well or better than media containing yeast extract.

6. Substances such as vitamin B_1, which are necessary in minute amounts for the growth of the isolated root but which are normally supplied by some other portion of the intact plant, are carriers of a growth correlation and hence are to be regarded as phytohormones.

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EFFECT OF INDOLEACETIC ACID ON GROWTH AND CHEMICAL COMPOSITION OF ETIOLATED BEAN PLANTS[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 483

JOHN W. MITCHELL AND WILLIAM E. MARTIN

(WITH FOUR FIGURES)

Introduction

In connection with recent investigations which show that hormones have a marked effect upon the morphological and histological development of certain plants (**2**), it was considered of interest to study the effect of 3 per cent β-indoleacetic acid on the chemical composition of plants treated with it. To facilitate such a study several thousand bean plants were grown in the dark. The synthesis of organic foods in addition to those already present, which might complicate the study, was eliminated in this way. Some of the plants were treated with indoleacetic acid and others were used as controls. The wet weight, dry weight, volume, sugar, starch, and nitrogen content of various portions of the treated and untreated plants were compared at the beginning and end of a four day period following treatment.

Investigation

MATERIALS AND TREATMENT.—Kidney beans, *Phaseolus vulgaris*, were used in the experiments. A great number of seeds were carefully selected for uniformity and then weighed individually, rejecting all that varied more than 50 mg. from the mean. In each experiment 1050 seeds were planted at a uniform depth in unsterilized quartz sand contained in fifty 12 inch clay pots. They were watered with warm tap water and placed in the dark at 80° F. with a relative humidity of 70–80 per cent saturation.

The plants were usually above the sand level on the third day

[1] This investigation was aided in part by a grant to the University of Chicago from the Rockefeller Foundation.

after planting, and on the fourth day the seed coats were removed. The cotyledons and epicotyls were thus freed so that further growth was not impeded by the mechanical obstruction of the seed coats. By the following day the plants were usually 6–7 inches high and the first internode from 0.5 to 1 inch in length.

Seven to eight hundred uniform plants were then selected for treatment. One third of these were treated by spreading approximately 0.05 cc. of 3 per cent β-indoleacetic acid lanolin mixture on the two opposite sides of the first internode. In this way about one third of a square centimeter of epidermis was covered with paste. The remaining two thirds of the plants were treated in a similar way by placing an equal amount of pure lanolin on the first internode. One half of the latter group was harvested immediately as initial controls, while the remaining plants, composed of an equal number of controls and treated, were allowed to remain in the dark under controlled conditions for four days, and then harvested.

PHYSICAL MEASUREMENTS.—At harvest the plants were first washed free from most of the sand, then dipped into a saturated solution of sodium chloride to loosen the remaining sand adhering to the roots. The plants were washed carefully and divided into the following fractions: (1) roots, (2) hypocotyls, (3) cotyledons, (4) first internodes, (5) second internodes together with the petioles and leaves.

The fresh weight of each fraction, representing the parts of approximately 250 plants, was then recorded and the volume measured by the displacement of water. In this way both the fresh weight and the volume were determined for each of the five fractions.

Immediately following these determinations the fractions were chopped finely and placed in a well ventilated drying oven at 80° C. for 20 hours. The dry weight of each fraction was then determined by weighing the samples in large weighing bottles on an analytical balance.

In all experiments the results were obtained from samples representing at least 225 plants. Preliminary experiments showed that 225 plants were a sufficient number to give accurate results. As a matter of convenience, some of the data are presented on the basis of 100 plants.

CHEMICAL METHODS.—Prior to analysis, all samples were ground by means of a coffee mill and then in a ball mill until the material would pass through an 80 mesh sieve. The ground material was then redried at 80° C. before samples were taken for analysis. The soluble sugars were removed from weighed samples of the dry powder by means of repeated extractions with 80 per cent alcohol, seven such extractions sufficing to remove the sugars and obtain a negative alpha naphthol carbohydrate test.

The extract so obtained was evaporated on a steam bath, and the alcohol replaced with distilled water. This aqueous extract was then cleared and the sucrose hydrolyzed by invertase, according to the procedure outlined by LOOMIS and SHULL (3). Following inversion, the solutions were made to volume and sugars determined according to the method of PHILLIPS (5).

The starch remaining in the residue after alcoholic extraction was hydrolyzed by fresh saliva as outlined by LOOMIS and SHULL, and the sugar in the final solution determined by the method of PHILLIPS. Starch values so obtained were expressed as glucose.

The total nitrogen content of the dry powders from the several regions of the plants was determined by the Gunning method, modified to include nitrates as described in the Official Methods (1). At the end of the experiment in both treated and untreated plants considerable quantities of nitrate nitrogen were found, especially in the hypocotyls. The amount present was considerably greater than the quantity which could have been supplied through the use of tap water.

Results and discussion

MORPHOLOGICAL OBSERVATIONS.—The first apparent reaction of plants treated with indoleacetic acid occurred within 24 hours after treatment, and was evidenced by a marked bending of the hypocotyls at a point about 2–4 inches below the cotyledons. This reaction was only temporary, and within 96 hours the hypocotyls of treated plants were nearly straight. Noticeable swellings of the first internodes occurred at the points where indoleacetic acid was applied, and in many cases the hypocotyls became enlarged immediately below the cotyledons within 36 hours after treatment. During the four days following treatment the first internodes (the point of treat-

ment) elongated very little, while those of control plants increased from an initial length of 1 inch up to 5 inches at the end of the experiment. The second internodes, petioles, and leaves of treated plants did not increase appreciably in size, while there was a marked increase in the size of these portions of control plants during the same period. There was little difference in the apparent size of the roots or hypocotyls of treated as compared with control plants, even at the end of the experiments. In many cases dense rows of roots were developed along the entire length of the hypocotyls. This inhibitive effect upon the elongation of plants grown in continuous darkness is in contrast to the effect observed in the case of plants similarly treated but grown under the conditions of alternating night and day that obtained in late May and early June in the greenhouse at Chicago (figs. 1, 2).

The histological responses and details shown by the treated regions closely resemble those shown by plants of comparable age grown under conditions of alternating light and darkness. The differences between treated and untreated plants grown continuously in darkness, however, are very great (figs. 3, 4).

WEIGHTS, VOLUMES, AND CHEMICAL COMPOSITION.—In general the analyses showed (1) that in the case of both treated and control plants, materials were rapidly moved from the cotyledons into other parts of the plants during the four days following treatment; (2) that a greater quantity of material was withdrawn from the cotyledons of control plants than was withdrawn from the cotyledons of treated plants during the same period; and (3) that indoleacetic acid as used in these experiments determined to a large extent the direction of flow of materials from the cotyledons.

Thus there was an appreciable decrease in the fresh and dry weight of the cotyledons of both control and treated plants, with an accompanying gain in the fresh and dry weight in at least some of the other parts of the plants (table 1). Starch, sugars, and nitrogenous compounds were transferred from the cotyledons into the different parts of the newly developed plants (table 2), so that some of these parts gained dry matter.

That a greater quantity of these materials was withdrawn from the cotyledons of control plants than was withdrawn from the

TABLE 1

SUMMARY OF EXPERIMENT 5 SHOWING EFFECT OF INDOLEACETIC ACID ON FRESH AND DRY WEIGHT OF DIFFERENT PARTS OF ETIOLATED BEAN SEEDLINGS; FIGURES EXPRESSED IN TERMS OF GRAMS PER 100 PLANTS

Region of plant	Initial control		Final control		Treated	
	Fresh weight	Dry weight	Fresh weight	Dry weight	Fresh weight	Dry weight
Roots	81.6	4.94	85.0	3.53	94.4	3.96
Hypocotyls	342.0	17.47	394.7	18.06	420.8	22.72
Cotyledons	91.2	26.21	42.7	7.43	48.6	9.57
First internodes	15.0	1.44	146.8	9.73	99.8	6.72
Second internodes and leaves	51.2	6.54	146.7	10.70	59.5	6.33
Total	581.0	56.60	815.9	49.48	723.1	49.30

TABLE 2

EFFECT OF INDOLEACETIC ACID ON DISTRIBUTION OF SOME CHEMICAL CONSTITUENTS OF ETIOLATED BEAN SEEDLINGS; VALUES OF DUPLICATE DETERMINATIONS EXPRESSED AS GRAMS PER 100 PLANTS

Region	Total nitrogen			Soluble sugars			Starch		
	Initial control	Final control	Treated	Initial control	Final control	Treated	Initial control	Final control	Treated
Roots	0.20	0.15	0.18	0.10	0.03	0.03	None	None	None
	0.20	0.15	0.18	0.10	0.03	0.03			
Hypocotyls	1.31	1.54	1.85	1.66	1.27	2.38	None	None	None
	1.31	1.51	1.85	1.63	1.38	2.39			
Cotyledons	1.20	0.18	0.35	1.73	0.63	0.84	7.17	0.14	0.74
	1.20	0.18	0.35	1.71	0.63	0.82	7.51	0.12	0.71
First internode	0.08	0.57	0.42	0.07	0.76	0.31	None	None	None
	0.08	0.58	0.43	0.07	0.77	0.32			
Second internode and leaves	0.49	0.88	0.54	0.04	0.58	0.17	None	None	None
	0.49	0.88	0.53	0.04	0.59	0.12			
Whole plant	3.28	3.31	3.34	3.57	3.33	3.70	7.34	0.13	0.73

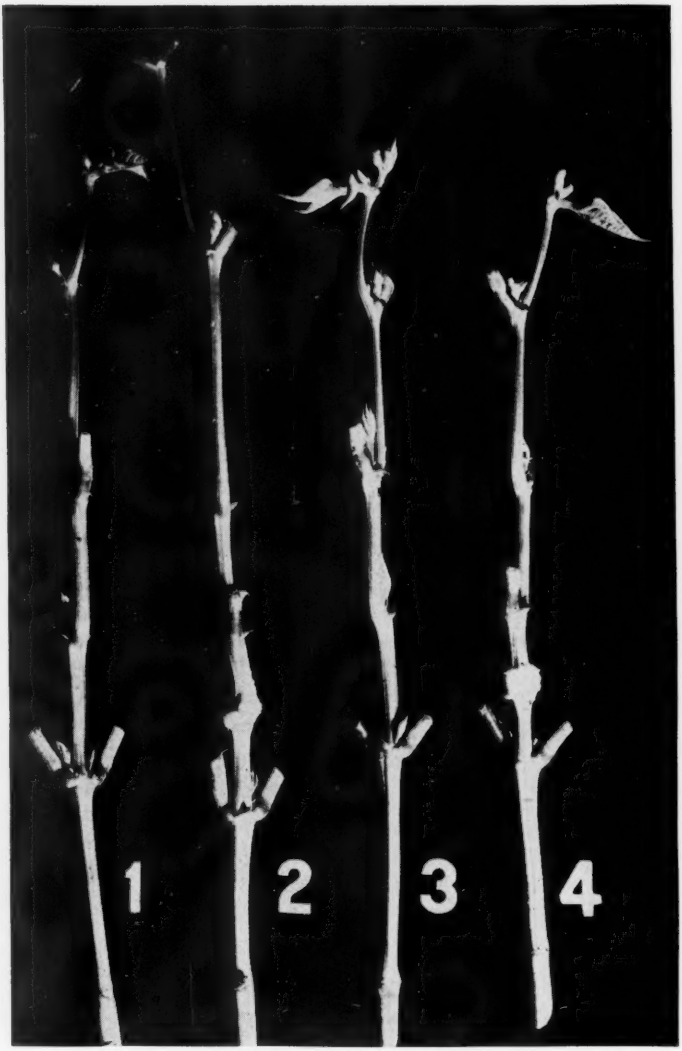

FIG. 1.—Stems of plants grown under natural light conditions: 2 and 4 treated laterally with a ring of 3 per cent indoleacetic acid lanolin mixture; 1 and 3 untreated. All thirteen days after treatment. Apart from formation of tumors and adventitious roots on treated stems, the two types were identical, with large green leaves and beginnings of flowering branches. From HAMNER and KRAUS (2).

FIG. 2.—Plants grown in continuous darkness four days after lateral application of lanolin mixture to first internode. After treatment there was but slight elongation of the first internode but marked increase in diameter, and many small lateral roots developed in the treated region. Histological details of the two types of stems are shown in figs. 3 and 4.

FIG. 3.—Transverse section through treated portion of plant grown in continuous darkness, four days after treatment. Pith cells living, but not meristematic; cambium and phloem tissues markedly proliferated; xylem not distorted; root differentiated from ray and phloem derivatives, and cells of pericycle and cortex disintegrating.

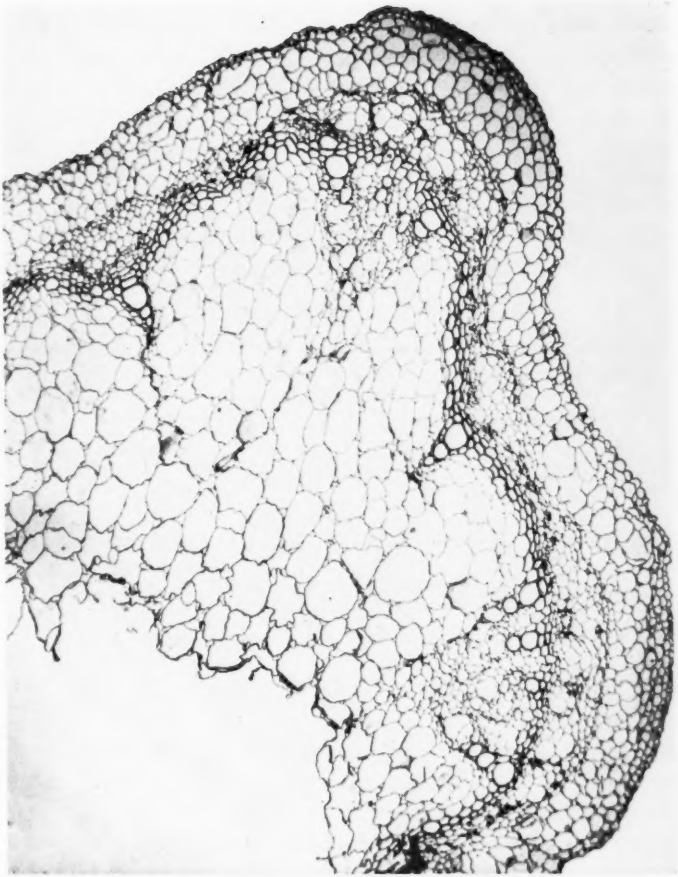

FIG. 4.—Transverse section through comparable portion of control plant grown under same environmental conditions as plants shown in fig. 3. Dead pith cells surrounding a central cavity; few derivatives have differentiated from the cambium, those of the xylem beginning to lignify; phloem parenchyma not proliferated; ray cells not meristematic; pericyclic, cortical, and epidermal cells not disrupted.

cotyledons of treated plants is evidenced, first by the loss of dry weight. In a study of about 2800 plants the results, expressed on the basis of 100 plants, show that the cotyledons of control plants decreased 69.20 gm. in dry weight during four days following treatment, while those of treated plants decreased only 64.26 gm. during the same time (table 3). Volume measurements of the cotyledons showed similar differences (table 4).

Further evidence of the inhibitory effect of indoleacetic acid upon the withdrawal of materials from the cotyledons is apparent from a

TABLE 3

SUMMARY OF EXPERIMENTS ON EFFECT OF INDOLEACETIC ACID ON DRY WEIGHT OF COTYLEDONS AND ENTIRE PLANTS; FIGURES BASED ON APPROXIMATELY 3700 PLANTS AND EXPRESSED AS GRAMS PER 100 PLANTS

Experiment	Cotyledons			Entire plant		
	Initial control	Final control	Treated	Initial control	Final control	Treated
1	.....	.....	.....	37.00	33.20	33.40
2	25.52	6.45	7.34	48.16	43.14	44.13
3	22.40	6.96	7.73	52.29	47.08	46.24
4	22.40	6.46	7.62	48.14	41.94	43.38
5	26.21	7.46	9.57	56.60	49.48	49.30
Total	96.53	27.33	32.27	242.19	214.84	216.45

consideration of the starch, sugar, and total nitrogen content of the cotyledons of treated as compared with those of control plants. On the basis of 100 plants, the cotyledons of the treated contained, at the end of the experiment, approximately 0.73 gm. of starch while those of control plants contained only 0.14 gm., expressed in terms of glucose. Cotyledons of 100 treated plants contained approximately 0.83 gm. of sugar and 0.35 gm. of nitrogen, while those of 100 control plants contained only 0.63 and 0.18 gm. of sugar and nitrogen respectively. The difference in the rate at which these materials were removed from the cotyledons, however, was not associated with a measurable difference in the rates of respiration of treated as compared with control plants, as will be mentioned later.

It is also evident that the application of indoleacetic acid appre-

ciably affected the amount of water absorbed by the plants as a whole. In the four days following treatment, control plants absorbed 62 per cent more water than did treated plants.

It has been pointed out that, following treatment, a large amount of the materials contained within the original seeds was transported from the cotyledons of both treated and control plants into the other parts of the plants. There were marked differences, however, between the places in which these materials were finally deposited in the control as compared with treated plants. Thus in experiment 5

TABLE 4

EFFECT OF INDOLEACETIC ACID ON VOLUME OF DIFFERENT PARTS OF ETIOLATED BEAN SEEDLINGS

Region	Initial control (cc.)	Final control (cc.)	Treated (cc.)
First internode*	15	167	110
Total above cotyledonary plate	.....	368	235
Cotyledons	.....	47	55
Total below cotyledonary plate	.....	534	557
Entire plant	637	902	782

* Portion of plant treated.

(table 1) that portion of control plants above the first internode gained approximately 64 per cent in dry weight, due to the deposit of materials transferred from the cotyledons during four days following treatment. In contrast to this, comparable parts of treated plants lost approximately 3 per cent of their original dry weight during the same time (table 1). The first internodes of treated plants gained in dry weight following treatment, but this gain was considerably less than the increase that was apparent in the first internodes of control plants. On the other hand, hypocotyls of treated plants gained more in dry weight following the application of indoleacetic acid than did similar parts of control plants. There was little difference in the dry weight of roots of treated as compared with control plants.

A comparison of the starch, sugar, and nitrogen content of the different parts of the plants leads to the same conclusion, namely, that indoleacetic acid applied to the first internode of etiolated plants inhibited the deposition of materials in the treated portions

and parts of the plants above the point of treatment. Materials which moved in the control plants from the cotyledons principally into the first internodes, second internodes, petioles, and leaves were deposited in the case of treated plants largely in the hypocotyls and first internodes. The volume of that portion of control plants above the cotyledons increased much more than did the volume of the same portion of treated plants following the application of indoleacetic acid (table 4). Transverse sections made through the treated portion of etiolated plants at the end of the experiment showed no apparent evidence of mechanical blockage or inhibition of development of the conductive tissues.

Although indoleacetic acid caused a marked difference in the morphological and histological development and in the rate and direction of transport of carbohydrate and nitrogenous compounds of the plants, it did not greatly affect their rate of respiration. Thus data collected from a study of approximately 3600 plants showed that the controls decreased 5.47 gm. in dry matter per 100 plants, due to respiration during the four days following treatment. The same number of treated plants lost 5.14 gm. during the same period. The difference in these values is not considered significant as it falls within the limits of experimental error.

In preliminary experiments the amount of carbon dioxide respired by treated and untreated etiolated beans was measured by the method described by MITCHELL (4). No significant differences were noted in the amount of carbon dioxide respired by the treated as compared with the untreated plants during a period of seven days.

Summary

1. Three per cent β-indoleacetic acid lanolin mixture applied to the first internode of etiolated bean seedlings caused the formation of galls at the point of application and the development of roots in the galls, and in many cases dense rows of roots developed the entire length of the hypocotyls.

2. The first internodes, second internodes, petioles, and leaves of treated plants increased less in volume, fresh weight, dry weight, and length than did corresponding parts of control plants.

3. The histological responses shown by treated regions of plants

grown in continuous darkness closely resemble those shown by plants of comparable age grown under alternating light and darkness, as reported by other investigators. Histological differences between treated and untreated plants grown in continuous darkness were very great.

4. The application of indoleacetic acid to the first internode of etiolated plants retarded the transport of materials from the cotyledons and also the uptake of water by the plants.

5. Analyses of treated and untreated plants that included the determination of fresh weight, dry weight, volume, starch, sugar, and nitrogen contained in different portions of the plants showed that indoleacetic acid greatly affected the direction of transport of materials from the cotyledons. Materials were conducted from the cotyledons upward only as far as the first internode (the point of treatment) in the case of treated plants. At the end of the experiments, transverse sections through the treated portion of plants grown in the dark showed no apparent evidence of mechanical blockage or inhibition of development of the conductive tissues.

6. No significant differences were observed in the amount of dry matter respired by treated as compared with control plants.

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PARTHENOCARPIC FRUITS INDUCED BY SPRAYING WITH GROWTH PROMOTING COMPOUNDS

F. E. GARDNER AND PAUL C. MARTH

(WITH FOUR FIGURES)

Introduction

Parthenocarpy occurs naturally in a number of plant species and by a variety of means has been induced artificially in others. Recently GUSTAFSON (2) obtained fruit development in several species which normally do not exhibit parthenocarpy by applying lanolin mixtures of growth promoting compounds on the styles, which had first been cut off close to the ovaries. HAGEMANN (3) also obtained parthenocarpic fruits in the case of *Gladiolus* by applying to the stigmas indoleacetic acid in a lanolin paste. The present report is an account of experiments in producing parthenocarpic fruits by means of spraying the blossoms with aqueous solutions of several well known growth promoting compounds. It is concerned chiefly with the American holly, *Ilex opaca*, but includes also results with the strawberry, the apple, and the grape.

The American holly is particularly well adapted for experiments on parthenocarpy in that it is dioecious, and the pistillate plants, which of course require no prior emasculation, can easily be protected from chance pollination. Moreover no development of the fruit, such as is common in unpollinated cucurbits, has previously been observed to occur in *Ilex opaca* without pollination, despite frequent attempts to induce fruit setting artificially. In some species, however, for example *I. cornuta*, parthenocarpy is not uncommon.

Experimental work on the various steps necessary in the production of small, fruit bearing plants of the American holly has been in progress for several years. Such plants, with their attractive green leaves and contrasting red fruits, are of value to the florist during the Christmas season. One of the laborious steps in the production of good plants has been hand pollination in order to insure a full set of

"berries."[1] In the search for an easier and less time consuming method of insuring fruit setting, unsuccessful attempts have been made to bring about parthenocarpy by dusting the open pistillate flowers with irritants, such as sulphur. Miscellaneous pollens, including that from the rose, daisy, iris, and dandelion, have also been applied but no fruits resulted. The application of holly pollen, however, normally resulted in 100 per cent fruit set.

Investigation

The holly plants used in the experiments on parthenocarpy which follow were propagated in September, 1936, from a single large pistillate tree. When holly trees are of bearing age the entire new shoot structure with its flower primordia is determined in the buds during the previous summer. The flush of growth made in the spring is therefore simply the unfolding and development of structures already preformed and is accomplished in two or three weeks. Ordinarily there is no further shoot elongation during the year, except under certain conditions to be mentioned later in this report.

When well rooted, the holly cuttings were potted and placed in a protected coldframe over winter in order to insure a normal termination of the rest period. In the spring they were moved to the greenhouse, where several inches of new growth was produced, and on this the flowers were borne. When in bloom, the plants were sprayed with dilute aqueous solutions of the following four synthetic compounds: indoleacetic, indolepropionic, indolebutyric, and naphthaleneacetic acids.

No alteration of the floral parts, such as shortening of the style, was deemed necessary in the case of holly, which has a broad stigma and an extremely short style (fig. 1). This characteristic may be an important factor in the parthenocarpic response obtained with the growth promoting compounds.

Spraying of blossoms of different ages.—Under greenhouse conditions, the pistillate holly flower remains open about one week, after which time the corolla withers and the pistil and pedicel become yellow before abscission. Before testing the relative effectiveness of various growth substances in causing parthenocarpy, it was desired

[1] Botanically the holly fruit is a compound drupe.

to determine the response of flowers of different ages, since this might be an important consideration in subsequent comparisons. Accordingly some plants were given a single spraying of 0.04 per cent indoleacetic acid in water before the flowers were open; that is, while the four petals of the corolla still completely covered the pistil. Other

FIG. 1.—Left: pistillate plant of *Ilex opaca* showing flowers borne singly, broad stigmas, short styles, and anthers entirely devoid of pollen. Lowest flowers denote approximate point where current growth begins. Right: staminate plant showing flowers borne in groups (usually of three), absence of pistils, but well developed anthers full of pollen.

plants were sprayed when the flowers had been open for one, three, five, and seven days respectively. All flowers which were open so that the spray could reach the pistil, responded equally well in development of parthenocarpic fruits. Flowers sprayed in the bud stage failed entirely to develop fruit, however, indicating that the stimulus is best transmitted through the pistil rather than through the heavy cuticle of the pedicel or other organs.

Although holly flowers remain in bloom for approximately a week, at any time during which period they are equally responsive to applications of indoleacetic acid, they also responded, at least in the case of naphthaleneacetic acid, several days after the corolla had completely withered and the flowers were ready to fall. Several plants were allowed to reach this stage in flowering and then sprayed with an aqueous solution of 0.006 per cent naphthaleneacetic acid. The pedicels and pistils had become yellow, giving the impression that the stigmas were no longer receptive. Within two days after spraying, however, the pedicels and pistils became green in color and fruits were subsequently developed.

COMPARATIVE EFFECTIVENESS OF SEVERAL GROWTH SUBSTANCES.—For these comparative tests sets of plants were selected having approximately the same number of open flowers. In order to facilitate the recording of number of flowers and fruits set per plant, all unopened flowers were first removed. The percentages of flowers which set fruit, shown in table 1, are based on from thirty to thirty-six flowers about equally distributed on four plants for each concentration shown. The four growth substances indicated were applied only once, care being taken that every flower was thoroughly sprayed.

The work of AVERY, BURKHOLDER, and CREIGHTON (1), together with that of ZIMMERMAN and HITCHCOCK (4), shows that the relative effectiveness of the growth promoting compounds in one plant response may be very different if measured in terms of some other reaction of the plant. To the evidence already available on the relative potency of the growth promoting substances in the various plant responses heretofore studied can now be added information relative to their effectiveness in parthenocarpy.

It is apparent from table 1 that, of the four compounds used, naphthaleneacetic acid was by far the most effective, in terms of concentration, in producing parthenocarpy in holly. Even at a dilution of 1 part per million an appreciable number of flowers set fruit. It is difficult to give the other three compounds a relative rating on the basis of the data presented, inasmuch as 100 per cent fruit set was not obtained with either indoleacetic or indolepropionic acids, yet both compounds caused some fruit setting at appreciably lower con-

centrations than did indolebutyric acid. It is probable that other factors, such as the rapidity of fruit development and the percentage of fruit which later abscissed, should also be considered along with the percentage set, in any attempt to evaluate the relative effectiveness of these three compounds in causing parthenocarpy. These factors are to be discussed presently.

The three highest concentrations of indolebutyric acid which resulted in 100 per cent fruit set also caused marked epinasty of the

TABLE 1

PERCENTAGE FRUIT SET IN RELATION TO EACH OF FOUR COMPOUNDS IN AQUEOUS SOLUTION OF VARIOUS CONCENTRATIONS

PERCENTAGE CONCENTRATION	PERCENTAGE SET			
	INDOLE-ACETIC	INDOLE-BUTYRIC	INDOLE-PROPIONIC	NAPHTHALENEACETIC
0.1	84.8	100	41.9	.....
0.08	53.6	100	65.6	.....
0.06	64.3	100	68.9	.....
0.04	54.5	60.6	54.8	.....
0.02	38.2	21.2	32.2	.....
0.01	9.0	0	22.5	100
0.008	3.2	0	0	100
0.006	3.0	0	3.1	100
0.004	6.0	0	12.1	97.2
0.002	0	0	.....	74.2
0.001	.....	0	.....	52.8
0.0008	.....	.....	.....	30.0
0.0005	.....	.....	.....	16.6
0.0003	.....	.....	.....	23.3
0.0001	.....	.....	.....	10.7

leaves. This was the only compound, and then only at the higher concentrations, which caused any observable disturbance of the foliage.

INFLUENCE OF REPEATED SPRAYINGS.—In the preceding comparison of the effectiveness of the several compounds, only one application of the spray was made, on the supposition that repeated sprayings might possibly interfere with an evaluation of the various concentrations tried. To determine the effect of repeated stimulus, however, the indoleacetic acid concentration series was applied four times in another group of plants at intervals of 24 hours, to compare

the results with those of a single application. Table 2 shows this comparison, the results with the single spraying being the same data appearing in table 1.

It is evident from table 2 that the repeated stimulus of successive sprayings with low concentrations is much more effective in parthenocarpy than single applications. In the practical use of these compounds in producing fruit on holly, successive sprayings would be necessary in that the blossoms are not all open at one time.

DEVELOPMENT OF PARTHENOCARPIC FRUITS.—Not only was naphthaleneacetic acid most effective at the lower concentrations in causing a high percentage of fruit setting but also in the rapidity of

TABLE 2

PERCENTAGE OF FRUIT SET RESULTING FROM SINGLE VERSUS REPEATED SPRAYS OF INDOLEACETIC ACID

No. of sprayings	Percentage concentrations of indoleacetic acid					
	0.02	0.01	0.008	0.006	0.004	0.002
1	38.2	9.0	3.2	3.0	6.0	0
4	79.4	36.3	30.3	12.1	17.6	0

its action in bringing about a greening of the pedicels and ovaries and a prompt swelling of the latter. Within two days after spraying with naphthaleneacetic acid development of the ovaries was easily discernible. This stimulation was much more rapid than that following pollination. The relative rapidity of reaction to the other acids, in descending order, was indolebutyric, indoleacetic, and indolepropionic. In the case of indolepropionic acid the observable initial development of the fruit was much slower than that in the case of pollination, and markedly slower than any of the other compounds.

Following pollination, approximate mature size of holly fruits is attained in seven or eight weeks. To all outward appearances the parthenocarpic fruits are like those developed following pollination, with the exception that, in the case of naphthaleneacetic acid, the fruits continue to be much greener in color and somewhat larger in

size (fig. 2). Data on the ripening of the fruits and the development of the red color following chilling weather is still to be obtained.

ABSCISSION RELATED TO SECOND GROWTH.—It was mentioned earlier that with bearing holly trees only one flush of growth normally occurs. With young seedlings and rooted cuttings, however, a second and even third flush is not uncommon if conditions for growth are very favorable prior to maturation of the terminal bud. This

FIG. 2.—Left: pistillate plant eight weeks after pollination. Right: similar plant sprayed with 0.01 per cent naphthaleneacetic acid.

second flush of growth, amounting to as much as 5–10 inches, occurred in many of the experimental plants, both in the pollinated and the sprayed lots (fig. 3). The number of sprayed plants making a second growth differed with the growth substance used and with the concentration. The data in table 3, showing the percentages of plants making this growth, are based on all concentrations applied and without regard to the set of fruit. They are not strictly comparable, therefore, since the concentrations of all four compounds

are not in identical ranges. The percentage of all fruits to drop is, however, closely associated with the percentage of all plants making second growth. Every sprayed plant which made a second growth

FIG. 3.—Left: pollinated plant which made a second flush of growth but held its fruit. Right: plant sprayed with indoleacetic acid which had eight fruits which abscissed when second flush of growth occurred. Approximate upper half of entire stem in each plant is of second flush growth.

dropped all its fruit. On the other hand, none of the pollinated plants dropped their fruit although all made a second growth. This

lack of abscission in the case of the pollinated fruits is probably associated with the development of the embryos within their seeds. Embryos are not present in the parthenocarpic fruits.

The inhibiting effect of the sprays on second growth was most marked in the case of naphthaleneacetic and indolebutyric acids in the higher concentrations used. As apparent from table 3, indolepropionic acid had very little influence in this respect.

FRUITS SET BY APPLYING INDOLEACETIC ACID TO SOIL.—In addition to parthenocarpy obtained by spraying, fruits were also produced by watering the soil around the roots of holly plants with rela-

TABLE 3

FRUIT ABSCISSION AND SECOND GROWTH OF HOLLY

TREATMENT	PERCENTAGE FRUITS DROPPED	PERCENTAGE PLANTS MAKING SECOND GROWTH
Naphthaleneacetic	25.5	36.3
Indoleacetic	40.4	56.8
Indolebutyric	51.8	53.5
Indolepropionic	95.7	87.5
Pollinated	0.0	100.0

tively concentrated solutions of indoleacetic acid during full bloom. A 0.15 per cent solution added in sufficient amounts on two successive waterings so that considerable drainage from the pots took place caused a number of flowers to set fruit. Relatively few plants were used in this experiment and no record was secured of the percentage set. Indoleacetic acid was the only compound applied in this way. The first trials with concentrations ranging from 0.0025 to 0.02 per cent were without effect. A concentration of 0.15 per cent produced no apparent injury and no epinasty.

Fruit was also set on some plants with indoleacetic acid by introducing small quantities of the dry powder into holes in the stem made with a small nail.

PARTHENOCARPIC STRAWBERRIES.—Individual potted plants of a strictly pistillate strawberry selection, protected in the greenhouse against chance pollination, were sprayed during a portion of their

blooming period with indoleacetic acid in concentrations of 0.1, 0.05, 0.025, 0.01, and 0.005 per cent respectively. All of these concentrations resulted in many of the blossoms producing apparently normal achenes which, upon subsequent examination, proved to be devoid of embryos. With the 0.05 and 0.1 per cent concentrations a number of the receptacles developed and ripened into apparently normal fruits (fig. 4). Never more than one fruit of an inflorescence, however, developed completely. In the lower concentrations the receptacles made only a slight initial growth, which soon ceased, although achenes usually developed. The receptacles or achenes of unsprayed flowers made no development.

Although some of the parthenocarpic achenes were cut open for examination, the majority, several hundred in all, were planted to ascertain whether any viable embryos were present. Only one rather weak seedling was obtained, which is still alive and is to be examined later for chromosome abnormalities. Achenes from pollinated fruits of the same strawberry selection germinated freely.

EXPERIMENTS WITH APPLE AND GRAPE.—Orchard trees of the Starking apple, a self-sterile variety, were protected from cross pollination and sprayed, when in full bloom, with solutions of indoleacetic acid ranging in concentrations from 0.01 to 0.06 per cent, but no fruits developed. In the higher concentrations used, marked injury to the pistils occurred, and the negative results were therefore not attributed to insufficient concentration, but tentatively to the relatively long style of the apple, through which length the stimulus of the growth substance might have some difficulty in passing. No attempts were made with the styles shortened by cutting.

The Brighton grape, which is self unfruitful, likewise failed to respond to naphthaleneacetic acid in concentrations ranging from 0.0005 to 0.01 per cent. This range was arbitrarily selected, and having failed, there was no opportunity to try stronger concentrations since the flowering period had passed. Since no serious injury occurred at the highest concentration used, it is thought that a still stronger solution might have resulted in parthenocarpy. The grape, like the holly, has a very short style.

It is evident from the results reported here that not all plants can

FIG. 4.—Parthenocarpic strawberry fruits. Upper: flowers sprayed with 0.1 per cent indoleacetic acid, which also caused lengthening and twisting of pedicels. Lower: fully developed parthenocarpic fruit of good size. Achenes which appear normal are empty. Note that never more than one fruit per inflorescence develops.

be expected to respond as readily as the holly to spraying with these compounds. Entirely apart from scientific interest, however, the results with holly do constitute an example of the practical value of the use of growth promoting compounds in effecting fruit setting.

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EFFECTS OF SUDDEN CHANGES OF TEMPERATURE ON ELONGATION RATE OF PRIMARY ROOT

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(WITH ONE FIGURE)

Introduction

ASKENASY (1), in a study of the effects of temperature upon growth, found that the roots of maize seedlings did not elongate at temperatures of 3° to 9° C. If roots which had been exposed to these low temperatures were returned to 20° C., they usually elongated more slowly than the controls for several days. ASKENASY noted that sudden changes of temperature produced a different effect from gradual changes. LEHENBAUER (8) gave data which supported this conclusion. The writer began an investigation of the effects of sudden changes of temperature on the elongation rate of roots under the direction of Professor CHARLES F. HOTTES at the University of Illinois. This paper reports the results of a continuation of this study.

Material and methods

Seedlings of *Phaseolus vulgaris*, Burpee's stringless greenpod bean, were used. The seeds were planted on moist, porous clay blocks in galvanized iron germinating pans. As soon as the seeds germinated the seedlings were removed, and all seedlings which did not possess straight roots and those growing from embryos broken in milling were discarded. The seeds were placed in baskets with the primary root extending into a pan of tap water. A pan of similar dimensions was inverted over the plants. The seedlings were grown in the constant temperature cases and kept at a uniform temperature.

As soon as a majority of the primary roots were 6 cm. long, their length was measured with a millimeter ruler and the length of each root recorded. Each seedling was labeled. After all the seedlings of a group were measured and labeled the roots of the seedlings were exposed suddenly to low temperatures.

A refrigerating unit was used for chilling. Large test tubes, filled with tap water, were extended into the brine. The roots were ex-

tended through perforated corks in the necks of the test tubes, about 1 cm. of the root tip extending into the cold water. The same type of result was obtained when the entire primary root extended into the water. A glass was inverted over the stems and leaves to prevent excessive transpiration. The roots were chilled for a definite period of time, and the temperature of the water in the test tubes was recorded with a maximum-minimum thermometer.

When seedlings were removed after chilling, they were placed in water of the temperature at which they were growing before chilling, and their growth was continued in the temperature case. At 24 or 48 hour intervals the lengths of the roots were measured. All measurements were taken at 8 A.M. The tap water in the pans was changed each time the roots were measured.

Experimental data

Effect of period of chilling upon elongation rate of root

Bean seedlings grown at 20° C. were sorted into groups of twenty-five having uniform range of length and elongation rate (for 24 hours before chilling). The rate of elongation for each group for the 24 hour period before chilling was 0.72–0.76 cm. / 24 hours / root. These groups were exposed to low temperatures 24 hours before the primary roots reached the first period of greatest elongation in the S-shaped curve of elongation. The roots were chilled at 9 A.M. to 3° C. for varying periods of time. Some of the roots were chilled for 3 minutes; some for 10 and 20 minutes; and others for 30, 40, 50, 60, 70, 80, 90, and 120 minutes. It was found that chilling for 3 minutes produced an accelerated elongation rate of the root during the following 24 hours (fig. 1). During the following 24 hour period the elongation rate of these roots was low. Roots which were chilled for 10 minutes were retarded in their elongation rate during the first 24 hour period after chilling. During the fifth 24 hour period after chilling they elongated slightly more rapidly than did the roots of the control seedlings. Those chilled 20, 30, 40, and 50 minutes elongated less rapidly than did the control roots.

The roots chilled 70 minutes elongated more rapidly than those chilled 10 or 50 minutes. Numerous other experiments confirmed this fact. Chilling a longer period of time must produce some ad-

justment in the protoplasm of the root. After this change has taken place the root can elongate more rapidly when transferred to 20° C. No root chilled 70 minutes elongated as rapidly as the roots of the control seedlings.

The roots chilled 90 minutes elongated less rapidly than those chilled 70 minutes. Those chilled 120 minutes elongated more rapidly than those chilled 90 minutes, but not so rapidly as those chilled 70 minutes. The nature of the adjustment which must be made by these cells has not yet been studied.

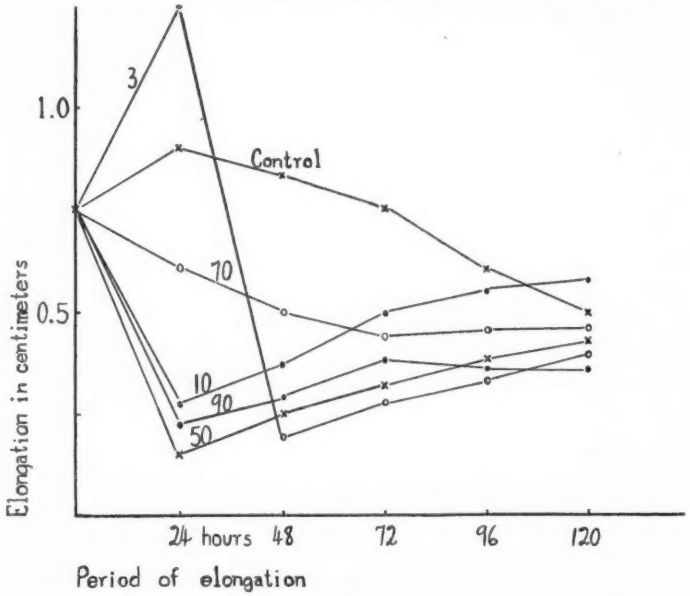

FIG. 1.—Effect of period of time roots are chilled on their elongation rate when returned to 20° C. Seedlings grown at 20° C.; roots chilled to 3° C. for various periods of time and again grown at 20° C. Hours after chilling are plotted along abscissa; increase in length for each 24 hour period after chilling is recorded along ordinate. Number of minutes each group was chilled shown on the curve for each group.

EFFECT OF TIME OF DAY ROOTS ARE CHILLED ON ELONGATION RATE

Bean seedlings were grown at 20° C. and the primary roots exposed to 3° C. for 10, 20, 30, and 40 minutes. The roots of twenty-

five seedlings were chilled at 8 A.M. for 10 minutes. The same number were chilled at 8 A.M. for 20, 30, and 40 minutes. Similar groups were chilled at 9 A.M., 10:30 A.M., 12:10 P.M., 1 P.M., and 3 P.M. These seedlings were placed under uniform conditions in the temperature case maintained at 20° C. There were some control seedlings in each pan. Measurements were taken at 48 hour intervals.

The roots chilled at 8 A.M. elongated about as rapidly as did the roots of the control seedlings during the following 48 hour period (table 1). The roots chilled at 9 A.M. elongated much more slowly during the first 48 hour period after chilling than those chilled at 8 A.M. The roots chilled at 10:30 A.M. elongated more rapidly than those chilled at 9 A.M. The elongation rate of those chilled at 12:10 was greater than that of those chilled at 10:30. The elongation rate of those chilled at 1 and at 3 P.M. was less than that of those chilled at 12:10 P.M.

Numerous experiments gave similar results. In each case the roots chilled at 9 A.M. and at 1 P.M. or 3 P.M. showed a retarded elongation rate. Those chilled at 8 A.M. or about 12 noon showed less retardation in elongation rate.

Periodicity of mitosis in bean root tips

Kellicott (**6**) found that elongation and mitotic activity in onion root tips grown at 20° C. show periodicity. Elongation was at its maximum when mitotic activity was at its minimum, and vice versa. The periods of greater mitotic activity in the onion root tips studied were at 11 and 1 P.M. Karsten (**5**), working with *Vicia faba*, *Zea mays*, and *Pisum sativum*, concluded that roots have no periodicity of mitotic activity, but that stems show periodicity to a marked degree. Laughlin (**7**) studied the periodicity of mitosis in onion root tips. He concluded that mitosis does show periodicity.

The root tips of seedlings grown at 25° C. were collected in formalin-acetic-alcohol solution (**3**). The root tip was dissected out with a small needle. The material thus dissected was stained as a smear with iron aceto-carmine stain (**3**). The number of cells in the various stages of mitosis were counted under an oil immersion lens. The number of cells in resting or vegetative stage, prophase, metaphase, anaphase, and telophase are recorded in table 2. The number after

TABLE 1

ELONGATION RATE OF ROOTS CHILLED AT DIFFERENT HOURS OF THE DAY

Hour chilled	Duration of chilling	Initial length of root (cm.)	Elongation rate (in cm.) during	
			First 48 hours after chilling	Second 48 hours after chilling
8 A.M.	Control.....	av.* 6.1 r.* 5.0–7.5	1.4 1.0–1.6	2.0 1.6–2.4
	10 minutes....	av. 6.0 r. 4.6–8.0	1.2 1.0–1.3	2.1 1.5–2.7
	20 minutes....	av. 6.0 r. 5.1–8.0	1.0 0.7–1.5	1.6 1.4–2.0
	30 minutes....	av. 5.9 r. 4.8–6.8	1.3 1.2–3.1	1.9 1.7–2.8
	40 minutes....	av. 6.0 r. 4.6–7.9	1.2 0.9–1.4	2.0 1.3–1–8
9 A.M.	Control.....	av. 6.0 r. 5.0–8.2	1.6 1.4–1.8	1.9 1.7–2.0
	10 minutes....	av. 6.2 r. 6.1–7.3	0.5 0.0–1.1	0.8 0.0–1.8
	20 minutes....	av. 5.8 r. 4.5–8.1	0.6 0.0–0.9	0.8 0.2–1.2
	30 minutes....	av. 6.2 r. 4.9–8.2	0.4 0.2–0.8	0.9 0.0–1.7
	40 minutes....	av. 6.1 r. 5.6–6.9	0.4 0.0–1.4	0.8 0.2–1.8
10:30 A.M.	Control.....	av. 6.0 r. 5.0–7.1	1.3 1.1–1.7	2.1 1.7–2.6
	10 minutes....	av. 6.0 r. 5.0–7.1	0.8 0.5–1.2	2.0 1.1–2.6
	20 minutes....	av. 6.0 r. 4.7–8.0	0.9 0.6–1.2	1.5 0.1–2.4
	30 minutes....	av. 5.7 r. 5.0–6.6	0.9 0.7–1.2	1.5 1.3–1.9
	40 minutes....	av. 6.3 r. 4.6–7.8	1.2 1.0–1.4	1.9 1.4–2.3

* Average and range for 25 roots.

TABLE 1—*Continued*

Hour chilled	Duration of chilling	Initial length of root (cm.)	Elongation rate (in cm.) during	
			First 48 hours after chilling	Second 48 hours after chilling
12:10 P.M.	Control.....	av. 6.1 r. 4.5–6.7	1.3 1.0–1.8	1.9 1.3–2.5
	10 minutes....	av. 6.4 r. 5.0–7.9	1.4 0.9–1.7	2.2 1.5–2.8
	20 minutes....	av. 5.8 r. 5.0–7.5	1.2 1.0–1.4	2.2 2.0–2.3
	30 minutes....	av. 6.0 r. 5.5–7.2	1.2 1.0–1.4	1.5 0.0–2.1
	40 minutes....	av. 6.3 r. 4.5–7.5	1.0 0.7–1.5	2.2 1.7–3.0
1 P.M.	Control.....	av. 6.3 r. 5.2–6.8	1.4 1.0–1.8	2.0 1.5–2.7
	10 minutes....	av. 6.6 r. 5.5–8.1	0.4 0.2–0.6	0.4 0.0–0.9
	20 minutes....	av. 5.9 r. 5.0–6.5	0.6 0.4–0.8	0.6 0.0–1.1
	30 minutes....	av. 6.3 r. 4.6–7.3	0.5 0.3–0.7	0.5 0.0–1.4
	40 minutes....	av. 5.9 r. 4.7–7.4	0.7 0.2–0.9	0.8 0.2–1.6
3 P.M.	Control.....	av. 6.0 r. 4.6–7.1	1.5 1.0–1.8	2.0 1.7–2.8
	10 minutes....	av. 6.3 r. 5.1–7.2	0.6 0.2–0.8	0.9 0.6–1.6
	20 minutes....	av. 6.2 r. 4.9–7.6	0.8 0.0–1.0	0.8 0.4–1.6
	30 minutes....	av. 6.0 r. 5.2–7.3	1.0 0.7–1.3	1.2 0.6–1.4
	40 minutes....	av. 5.9 r. 5.0–6.8	0.6 0.2–1.2	0.7 0.0–2.0

each stage indicates the equivalent of the stages counted by LAUGHLIN (7). There is little difference between the number of cells in mitosis at the various hours of the day. There are more cells in prophase at 9 than at 10 or 11 A.M. There are more cells in prophase again at 12 noon than at 1 P.M. The total number of prophases is greater at 9 A.M. than at 12 noon or 3 P.M. There are more cells in anaphase and telophase at 11 A.M. and at 2 P.M. than at other hours of the day.

The primary roots of seedlings of these groups were chilled to 3° C. for 30 minutes at 8, 9, 10, 11 A.M., 12 noon, 1, 2, and 3 P.M.

TABLE 2

NUMBER OF CELLS IN MITOSIS

TIME	STAGE IN MITOSIS (EQUIVALENT OF LAUGHLIN'S STAGES DESIGNATED BY NUMBER AFTER STAGE)									TOTAL NUMBER IN MITOSIS
	RESTING	EARLY (1, 2) PROPHASE	MIDDLE (3) PROPHASE	LATE (4) PROPHASE	METAPHASE (5)	ANAPHASE (6, 7)	LATE (8) ANAPHASE	EARLY (9) TELOPHASE	LATE (10) TELOPHASE	
8 A.M.	907	11	3	3	23	11	17	8	17	93
9	867	41	23	16	21	13	3	14	2	133
10	894	32	10	10	16	16	10	11	1	106
11	894	1	2	5	19	24	15	6	34	106
12M.	879	59	23	0	19	8	9	2	1	121
1 P.M.	918	19	20	0	17	3	6	5	12	82
2	905	8	4	8	10	14	13	7	31	95
3	901	18	12	0	30	13	11	14	1	89

They were chilled 48 hours before reaching the first period of greatest elongation in the S-shaped curve of growth, and then grown at 25° C. The rate of elongation of the primary roots of these seedlings is recorded in table 3. Roots chilled at 9 A.M., 12 noon, and 3 P.M. showed more retardation than those chilled at 8 and 11 A.M., or 2 P.M. It was found that the roots which showed a greater retardation of elongation rate after chilling were those which had been chilled when more cells were in prophase. ATKINSON (2) reported that pollen mother cells obtained during cold days in early spring were in prophase. No stages of division beyond prophase were found. SAX (10) found that nuclear and cell divisions in pollen mother cells and microspores of *Tradescantia* were disturbed by exposure to low and high temperatures.

TABLE 3

ELONGATION RATE OF ROOTS CHILLED AT DIFFERENT HOURS OF THE DAY

HOUR CHILLED	DURATION OF CHILLING	INITIAL LENGTH OF ROOT (CM.)	ELONGATION RATE (IN CM.) DURING	
			FIRST 48 HOURS AFTER CHILLING	SECOND 48 HOURS AFTER CHILLING
8 A.M.	Control.....	av.* 6.2 r.* 5.1–7.5	2.6 1.7–3.0	3.1 2.0–3.6
	30 minutes....	av. 6.0 r. 5.1–7.7	2.1 1.4–3.0	2.7 1.8–2.8
9	Control.....	av. 6.0 r. 5.0–7.1	2.8 1.6–2.8	3.0 1.8–3.2
	30 minutes....	av. 6.1 r. 5.6–7.1	1.0 0.5–2.1	1.8 0.0–3.6
10	Control.....	av. 6.1 r. 5.6–6.8	2.4 1.7–3.2	2.9 1.9–3.4
	30 minutes....	av. 6.0 r. 4.6–7.2	1.7 1.1–2.0	2.8 1.0–3.5
11	Control.....	av. 5.8 r. 5.0–6.5	2.5 1.8–2.8	2.9 2.1–3.3
	30 minutes....	av. 6.1 r. 5.1–7.2	2.7 1.5–3.0	3.0 2.1–3.4
12 NOON	Control.....	av. 5.9 r. 4.9–6.9	2.5 1.5–2.9	2.9 1.6–3.1
	30 minutes....	av. 6.0 r. 4.8–6.7	1.0 0.2–1.2	1.2 0.0–2.1
1 P.M.	Control.....	av. 5.8 r. 5.0–7.9	2.4 1.6–3.1	2.9 1.8–3.6
	30 minutes....	av. 5.9 r. 5.0–7.1	1.3 1.0–1.5	2.0 1.5–2.4
2	Control.....	av. 6.1 r. 5.1–7.2	2.5 1.5–2.8	2.8 1.9–3.4
	30 minutes....	av. 6.0 r. 4.7–6.8	2.0 1.6–2.5	2.5 1.7–3.1
3	Control.....	av. 6.2 r. 5.2–6.8	2.3 1.6–3.1	2.8 1.4–3.7
	30 minutes....	av. 6.1 r. 5.1–6.9	0.9 0.0–1.2	1.2 0.5–2.0

* Average and range for 20 roots.

It was noted that the turgidity of the cells varied in relation to the hour of the day the roots were chilled. Some roots were more turgid when removed from chilling than were other roots. Investigations of this relationship will be reported later. There are many factors which vary in cells with the mitotic cycle.

Effect of heating on elongation rate of roots

Seedlings were grown at 15° C. and their roots exposed to water maintained at 35° or 40° C. for 30 minutes. The seedlings were re-

TABLE 4

Effect of initial length and age of roots on elongation rate of roots after chilling

Roots chilled at 3°C. for 20 minutes; seedlings grown at 20°C.

Age (days since placed in temperature case)	Initial length (cm.)	Average elongation for 48 hours before chilling (cm.)*	Hour chilled	Average elongation for first 48 hours after chilling (cm.)*	Average elongation for second 48 hours after chilling (cm.)*
6.....	4.5–5.0	1.9	9 A.M.	0.5	0.6
			12 NOON	2.0	1.4
6.....	7.0–8.5	2.2	9 A.M.	0.8	0.7
			12 NOON	2.6	1.5
8.....	5.5–6.7	1.1	9 A.M.	0.1	0.3
			12 NOON	1.2	1.1
8.....	8.0–9.0	1.5	9 A.M.	0.8	1.0
			12 NOON	1.4	1.2

* Average elongation for 20 roots recorded.

turned to 15° C. and their elongation measured at regular intervals. Similar results to those reported for roots which were chilled were obtained. More marked results were produced by treating at 40° than at 35° C.

Effect of age of seedlings and their initial length on elongation rate after chilling

The initial length of the seedlings did not affect the type of response of the seedlings after chilling. The stage of elongation in the S-shaped curve of growth had little effect on the response to chilling. These data are recorded in table 4.

Discussion

Numerous investigators (**12, 6, 4, 5, 7**) have noted a rhythm in mitosis. KELLICOTT found that elongation and mitosis were periodic. The period of greater mitotic activity was the period of less elongation, and vice versa. When a cell is undergoing mitosis it is resting from its normal metabolic activities, and when it is metabolically active it does not undergo mitosis. The amount of food stored in the cell, the rate at which food is used, the oxygen consumption, and the water absorption must vary during the different stages of this growth cycle of the cell. Viscosity of the protoplasm is lowered during the early stages of mitosis. It rises during the later stages, and then drops to its original value (**11**). The turgor pressure also varies. In animal cells it has been found that reversible changes in surface tension, electrical polarization, permeability, and osmotic pressure take place during mitosis. The susceptibility of sea urchin eggs to heat and poisons is greater during mitosis. The period of greatest sensitivity is prophase. The sensitivity to poisons is less 35–45 minutes after cleavage (**9**).

At the present time it is impossible to state which factors are responsible for the results obtained. The data seem sufficient to conclude that the hour of day roots are exposed to low temperatures influences their elongation rate when transferred to 20° C. In control seedlings it was found that cells produced by mitotic activity started to elongate within the first 24 hours after their formation. If the mitotic activity of the embryonic cells of the root tip was disturbed by exposure to low temperatures, elongation during the following periods would be slower. Other changes, which occur periodically in the cells, may also influence the elongation rate after exposure to low temperatures.

Summary

1. The period of time roots were exposed to low temperatures influenced their elongation rate when returned to 20° C. After two or three 24 hour periods the elongation rate tended to return to the elongation rate of the controls.

2. The hour of the day at which root tips were exposed to low temperatures determined the elongation rate of the roots when trans-

ferred to 25° C. The roots chilled when more cells were in prophase were retarded in elongation rate. Cells formed by mitotic activity of the embryonic cells started to elongate within the first 24 hours after their formation. Other factors may influence the results obtained.

3. Exposing roots grown at 15° C. to high temperatures produced results similar to those produced in roots exposed to low temperatures.

4. The age of roots and their initial length did not affect the elongation rate of the roots which had been exposed to low temperatures.

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CURRENT LITERATURE

Phytohormones. By F. W. WENT and KENNETH V. THIMANN. New York: Macmillan Co., 1937. Pp. xi+294. 62 figs. $4.00.

The tone of this work is well expressed by the writers when they state, in relation to the question of priority of the discovery of the auxins, "We feel that the gradual unfolding of the current conceptions of the cooperation of different workers has made it impossible to credit any one person with such a discovery, and it is to be hoped that the reader of this book will gain the impression of a steady and collective advance rather than of individual contributions." And again, "There is danger, in any rapidly developing field, of an accumulation of unclassified facts and unproven theories which makes further development much less certain. In the field of phytohormones this is particularly unjustifiable, because the experimental procedure is relatively simple and the equipment necessary is not too elaborate. If the criteria of high-class experimental work continue to be observed, then we may look forward in the next few years to the rapid solution of a great many of the interesting problems of growth and development." Indeed, those who wish to follow the development of this interesting field may well applaud these expressed desires of the authors and trust that the present torrential rush toward priority, and above all, the formulation of theories and interpretation on less than meager data, may be slackened.

The details given in the chapter on the technique of auxin determination are direct and helpful without over-simplification of the process. The following six chapters embody an enumeration of a wide variety of experiments reported by many investigators, which deal with the formation and occurrence of auxins, their chemistry, their transport in the plant, and suggested correlations and interpretation of physiological processes and responses of plants on the basis of the phytohormone concept. Here are both the most and the least convincing parts of the book, for one feels strongly the great need of additional evidence, particularly such as would deal with the interrelationships of foods, nutrients, and hormones both from a qualitative and a quantitative viewpoint. Needed also is histological and cytological evidence. That presented seems extremely meager compared with the abundant references on generalized or gross responses. All these deficiencies, however, are in the field of phytohormone study. The authors could do no more than make these lacks obvious.

There is a comprehensive list of references to pertinent literature and helpful indexes to authors and subject matter. The book, assembled and written by two of the most active contributors to the field of study of phytohormones, has

directness of attack and frankness of analysis, both in relation to methodology and to interpretation of the results of experimentation. This treatise may well serve as one means toward stabilization of the subject.—E. J. KRAUS.

Studies on Wheat Grown under Constant Conditions. A Monograph on Growth. By H. VAN de SANDE-BAKHUYZEN. Stanford University, Food Research Institute, 1937. Pp. xvi+400. Figs. 27. $4.00.

A fundamental study of the growth of the wheat plant under controlled conditions has been made under the auspices of the Food Research Institute, and this, in many respects excellent, monograph presents the results of the investigation. There are thirty-six chapters grouped into seven main sections, which follow an introductory chapter. These seven sections bear the following titles: the growth curve in annual plants; methods and materials; general growth features; dry weight and moisture content of the different organs; dry weight and moisture of the standard plant; nitrogen and carbon of the organs; and nitrogen metabolism in relation to growth and development. In the collection of data, and in the interpretation of the materials of section VI, the author had the aid of E. P. GRIFFING and CARL L. ALSBERG. Summaries at the end of the various sections make it easy to get the gist of the report with a minimum effort.

Some of the information presented has been drawn from previously published investigations. The chapters on nitrogen balance, on the relation between respiration, nitrogen metabolism, and growth, and on aging in respect to external conditions are especially interesting.

In general, a very satisfactory beginning has been made in understanding the growth processes of a single important food plant. Naturally any work of this kind always leaves the impression that we still need much more information than the monograph can supply. It is a satisfaction to know that intensive and extensive research of this kind can be continued indefinitely, so that there will be an ever increasing knowledge of the nature of growth processes. All of the other prominent food plants deserve the same systematic investigation of their growth processes and responses.—C. A. SHULL.

Soil Conditions and Plant Growth. By SIR E. J. RUSSELL. London: Longmans, Green and Co., 1937. Pp. viii+655. Figs. 65. 21/0 net.

With only slight increase in size, the seventh edition of this masterpiece is now available. The author has incorporated much new material into the text, as the result of his visits to many European Agricultural Experiment Stations and his contacts with soil scientists who attended the Third International Congress of Soil Science at Oxford in 1935. The appendix of analytical methods has been omitted, and the bibliography shortened to conserve space. The volume is without doubt the most valuable single book on this subject in the English language.—C. A. SHULL.